Renin-angiotensin-aldosterone system and corticosteroids in heart failure

By

Yannis Tsorlalis MD

Submitted in fulfilment of the requirements for the degree of PhD

School of Medicine

College of Medical, Veterinary and Life Sciences

University of Glasgow

December 2012

List of Tables

List of Figures

Acknowledgement

I would like to acknowledge my supervisors Prof John McMurray and Prof John Connell for the opportunity to undertake this research and their expertise. Prof McMurray's vision and guidance have been invaluable. I am also grateful to Prof Robert Fraser for teaching me corticosteroid biochemistry and motivating me during my studies in Glasgow. I would like to thank Dr Marie Freel for all her advice and direction in the analysis of the steroid data.

I would like to acknowledge Dr Colette Jackson for the joint recruitment of a large heart failure population in Glasgow and the collection of patients' information, measurement and analysis of data in conjunction with the Microvolt T-wave alternans study.

The help of the nursing staff at the British Heart Foundation Glasgow Cardiovascular Research Centre and Glasgow Clinical Research Facility is much appreciated.

I would like to thank Ms Mary Ingram for measuring plasma corticosteroids for my studies. I would also like to thank Prof Eleanor Davies, Dr Gordon Inglis, Dr Scott MacKenzie, Dr Samantha Alvarez-Madrazo and Mrs Elaine Friel for their guidance and help with the genetic studies and laboratory techniques. A thank you to Mrs Christine Holloway for her time and patience in teaching me the art of genotyping. I am also grateful to the Biochemistry Department in the Royal Infirmary in Glasgow, especially Prof Michael Wallace, for the measurement of plasma renin concentration for my studies. Similarly, I would like to thank Dr Richard Spooner for the measurement of B-type natriuretic peptide in heart failure patients.

I would like to thank Dr Frances McManus for helping me to obtain the angiotensin II vials supply and for sharing ideas about the studies of the healthy subjects. I would also like to

thank the Pharmacy Production Unit and especially Graham Conkie regarding the preparation of the angiotensin II solutions used in these studies.

I would like also to thank Caroline Haig, Nicola Greenlaw and Prof Ian Ford for their guidance and help with the statistical analyses of the survival data in the current thesis.

I am grateful to Dr Sandosh Padmanabhan for his valuable advice about the genetic studies. I would also like to thank Tony Cunningham for calculating the left ventricular ejection fraction in patients with heart failure at the follow-up visit.

I am grateful to the patients of the NHS Greater Glasgow and Clyde and the healthy volunteers who gave willing to participate in this research.

I would like to express my gratitude to the Hellenic State Scholarships Foundation (I.K.Y) for funding my PhD in Scotland.

I am exceptionally grateful to my and Angelina's parents and friends Konstantinos, Christodoulos and Iliana for their constant encouragement. Finally, I cannot express my gratitude enough to my partner in life and wife Angelina for all her patience and endless support throughout my PhD studies.

Author’s declaration

I declare that the work presented in this thesis is, to the best of my knowledge and belief, original and my own work, unless specified otherwise in the text. This work has not been submitted previously for a higher degree. It was carried out under the supervision of Professor John McMurray and Professor John Connell at the British Heart Foundation Glasgow Cardiovascular Research Centre.

December 2012

Definitions/Abbreviations

11beta-HSD1	11beta-hydroxysteroid dehydrogenase 1
11beta-HSD2	11beta-hydroxysteroid dehydrogenase 2
ACE	Angiotensin-converting enzyme
ACTH	Adrenocorticotropic hormone
ADM	Adrenomedullin
AF	Atrial fibrillation
A-HeFT	African-American Heart Failure Trial
ALOFT	Aliskiren Observation of Heart Failure Treatment
ANOVA	Analysis of variance
ANP	Atrial natriuretic peptide
ARB	Angiotensin receptor blocker
ASTRONAUT	Aliskiren Trial on Acute Heart Failure Outcomes
AT_1	Angiotensin II type 1
AT_2	Angiotensin II type 2
BH4	Co-factor tetrahydrobiopterin
BHF	British Heart Foundation
BMI	Body mass index
BNP	B-type natriuretic peptide
Bpm	Beats per minute
BRIGHT	British Genetics of Hypertension
CHIF	Corticosteroid hormone-induced factor
CI	Confidence interval
CONSENSUS	Cooperative North Scandinavian Enalapril Survival Study
CRH	Corticotropin releasing hormone
CRP	C-reactive protein
CVA	Cerebrovascular accident
CYP11B1	11beta-hydroxylase gene
CYP11B2	Aldosterone synthase gene
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
DOC	11-Deoxycorticosterone
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
EMPHASIS	Eplerenone in Mild Patients Hospitalisation and Survival Study in Heart Failure
ENaC	Epithelial sodium channel
EPHESUS	Eplerenone Post Myocardial Infarction Heart Failure Efficacy and Survival Study
FBC	Full blood count
G6PD	Glucose-6-phosphate dehydrogenase

GILZ	Glucocorticoid induced leucine zipper protein
GR	Glucocorticoid receptor
GRI	Glasgow Royal Infirmary
GRAHF	Genetic Risk Assessment of Heart Failure in African-Americans
HDL	High density lipoprotein
HF	Heart failure
HFpSF	Heart failure with preserved systolic function
HFrSF	Heart failure with reduced systolic function
HPA	Hypothalamus-pituitary-adrenal
HPLC	High-performance liquid chromatography
HR	Hazard ratio
IC	Intron 2 conversion
Ica	Inward L-type calcium current
IQR	Interquartile range
ISD	Information services division
Ito	Transient outward potassium current
IV	Intravenous
JGA	Juxtaglomerular apparatus
LCMS	Liquid Chromatography Mass Spectrometry
LD	Linkage disequilibrium
LDL	Low density lipoprotein
LV	Left ventricular
LVEDD	Left ventricular end-diastolic diameter
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
LVSD	Left ventricular systolic dysfunction
MAP	Mean arterial pressure
MDRD	Modification of Diet in Renal Disease
MI	Myocardial infarction
MR	Mineralocorticoid receptor
Na^+- K^+-ATPase	Sodium-potassium ATPase
NADPH	Nicotinamide adenine dinucleotide phosphate
Nedd4-2	Neuronal precursor cells-expressed developmentally down-regulated protein 4-2
NF-Kappa B	Nuclear factor-kappa B
NO	Nitric oxide
NOS	Nitric oxide synthase
NYHA	New York Heart Association
O_2^-	Superoxide anion
OxLDL	Oxidised LDL
PCIP	Pro-collagen type I carboxy-terminal peptide
PIIINP	Pro-collagen type III amino-terminal peptide
PINP	Pro-collagen type I amino-terminal peptide
POMC	Proopiomelanocortin
PRA	Plasma renin activity

PRC	Plasma renin concentration
RAAS	Renin-angiotensin-aldosterone system
RAH	Royal Alexandra Hospital
RALES	Randomised Aldactone Evaluation Study
ROS	Reactive oxygen species
SAVE	Survival and Ventricular Enlargement
SBP	Systolic blood pressure
SD	Standard deviation
SF-1	Steroidogenic factor-1
SGK1	Serum glucocorticoid-regulated kinase 1
SNS	Sympathetic nervous system
SR	Sinus rhythm
StAR	Steroidogenic acute regulatory protein
TIA	Transient ischaemic attack
TOPCAT	Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist
TSH	Thyroid stimulating hormone
U&Es	Urea and electrolytes
VSMC	Vascular smooth muscle cell
WHO	World Health Organisation
WIG	Western Infirmary in Glasgow
ZF	Zona fasciculata
ZG	Zona glomerulosa
ZR	Zona reticularis

Summary

Greater renin-angiotensin-aldosterone system (RAAS) activity, as reflected by higher levels of renin and aldosterone, has been associated with worse prognosis in patients with chronic heart failure (HF). These findings provided the basis for clinical trials with RAAS inhibitors in these patients. Similarly, higher levels of cortisol have been correlated with worse outcomes in chronic HF. However, there is lack of information with respect to the activity of RAAS and glucocorticoid secretion in patients with decompensated HF. Is RAAS universally activated in patients with worsening HF? Are cortisol levels elevated in these patients? Furthermore, the prognostic importance of RAAS mediators and plasma glucocorticoid levels in patients with decompensated HF remains unknown.

Diuretic therapy is one of the initial therapeutic strategies in patients with decompensated HF and fluid congestion. Diuretics decrease the extracellular volume and suppress natriuretic peptide levels while they in parallel stimulate RAAS activity early after initiation of therapy. However, it is unknown if the dissociation between RAAS and natriuretic peptides after initiation of diuretic treatment persists over time. If that remains present in the long term, augmentation of the natriuretic peptide actions on top of RAAS inhibition might be of further benefit in patients with HF, given their suppressing effects on RAAS and sympathetic nervous system (SNS) and vasodilating and natriuretic properties.

The late steps in corticosteroid synthesis are mediated by aldosterone synthase for aldosterone and 11beta-hydroxylase for cortisol respectively. These enzymes are highly homologous and are encoded by genes that lie in tandem in chromosome 8 in humans. A

common single nucleotide polymorphism (-344T/C) in the promoter region of aldosterone synthase gene *(CYP11B2)* has been associated with aldosterone levels and the aldosterone to renin ratio in healthy subjects and patients with hypertension. Similar findings have been shown for another polymorphism in the same gene, the Intron 2 conversion (IC). Moreover, these polymorphisms have been correlated with the 11-deoxycortisol to cortisol ratio, which represents an index of 11beta-hydroxylase activity. In patients with severe HF of African-American origin -344T/C polymorphism has been associated with prognosis. However, the data with regards to the -344T/C polymorphism (and the IC) in relation to gluco- and mineralo-corticoid secretion and survival in patients with HF of Caucasian origin remains to be elucidated.

The hypothesis of the current thesis was that plasma levels of RAAS mediators and glucocorticoids are associated with markers of HF severity in patients with decompensated HF and in patients with stable HF. Moreover, the dissociation between RAAS activity and natriuretic peptides seen early after initiation of diuretic treatment persists in the medium to the long term. In addition, that higher levels of plasma renin, mineralo- and gluco- corticoids are associated with worse prognosis in patients with decompensated HF. Lastly, that *CYP11B2* polymorphisms -344T/C and IC are associated with mineralo- and gluco-corticoid secretion and survival in these patients. In order to test these hypotheses, 722 patients with decompensated HF were enrolled in the current studies. Of these, 453 surviving patients returned for the follow-up visit 4-6 weeks after discharge. All these patients had detailed clinical and biochemical phenotyping and additionally genotyping of the -344T/C and IC *CYP11B2* polymorphisms.

In this thesis, it was shown that levels of RAAS mediators, plasma renin concentration (PRC) and aldosterone, are not on average elevated during hospital admission in patients with decompensated HF. RAAS activity has been previously shown to be activated in patients with advanced congestive HF. However, high doses of diuretics were used and a significant proportion of patients were taking an aldosterone blocker in these studies. The results of the current study are in accordance with early studies with small numbers of untreated patients with congestive HF that reported normal or low levels of renin and aldosterone.

PRC and aldosterone levels were higher at follow-up compared with hospital admission likely due to a decline in the intravascular volume. In contrast, natriuretic peptide levels were lower at the follow-up visit and that is likely to contribute to the higher levels of RAAS mediators after discharge as these peptides exert suppressing effects on the RAAS and SNS.

Similarly to PRC and mineralocorticoid levels, glucocorticoid concentrations were within the normal range in patients with decompensated HF. Furthermore, it was shown for the first time that cortisol levels during admission are associated with clinical status and prognostic markers of HF, such as B-type natriuretic peptide (BNP) and troponin. That may represent an association reflecting the greater stress response due to HF severity. However, growing evidence supports that cortisol under conditions of altered intracellular redox state becomes a mineralocorticoid agonist and that might contribute to these associations. Overall, these findings call into question the “normal range” of cortisol levels in patients with HF.

Moreover, 11-deoxycortisol to cortisol ratio was shown to be lower in patients with left ventricular (LV) remodeling, lower blood pressure and greater RAAS activity during hospital

admission. 11-deoxycortisol to cortisol ratio represents an index of 11beta-hydroxylase, which is an enzyme regulated by adrenocorticotropic hormone (ACTH). Lower 11-deoxycortisol to cortisol ratio represents higher enzyme activity and reflects a state of chronic ACTH stimulation. These findings indicate that patients with features of worse HF are characterised by activation of the hypothalamus-pituitary-adrenal (HPA) axis.

PRC but not aldosterone was associated with an increased risk of all-cause mortality in patients with decompensated HF, even after adjustment for a combination of other variables shown to exert an independent prognostic value in the overall HF population. In contrast, cortisol was not an independent prognostic factor in patients with decompensated HF.

Lastly, no association was seen between aldosterone levels and -344T/C or IC polymorphism in the current study. *CYP11B2* -344TT genotype was associated with relative impairment of 11beta-hydroxylase, as reflected by the higher 11-deoxycortisol to cortisol ratio, in keeping with previous studies in healthy subjects and patients with hypertension. However, no association was found between *CYP11B2* polymorphisms and prognosis in the current studies.

1. Chapter One – General introduction

1.1 Epidemiology and pathophysiology of HF

1.1.1 Definition

HF is a complex clinical syndrome defined as an abnormality in the cardiac structure or function leading to heart inability to deliver oxygen to the tissues at a sufficient rate in relation to their metabolic needs (1). HF is characterised by symptoms such as breathlessness during exertion or at rest and fatigue and signs such as pulmonary congestion and peripheral oedema. It can present suddenly as acute HF, usually as a consequence of an acute coronary syndrome, or in a chronic fashion characterised by gradual worsening of HF symptoms and signs. Chronic HF can also be complicated by an acute on chronic deterioration requiring urgent treatment or hospitalisation due to decompensation. Depending on the ventricular systolic function, it can be also classified as HF with reduced systolic function (HFrSF) or as HF with preserved systolic function (HFpSF). The ejection fraction (EF), which refers to the ratio of stroke volume to end-diastolic ventricular volume, has been traditionally employed to describe HF according to systolic function. Although there is no clear cut-off, patients with HFrSF have left ventricular EF (LVEF) <35-40%; these are the patients who have been principally enrolled in the major trials in HF and gained benefit with regards to mortality risk reduction from the available therapeutic measures. On the other hand, patients with HFpSF have LVEF >40-45%; these are the patients with evidence of diastolic dysfunction and to date no treatments have improved clinical outcomes in this group of patients (2).

1.1.2 Epidemiology

The prevalence of HF in developed countries is estimated to be approximately 1-2% of the adult population (3). HF is predominantly a syndrome of the elderly and its prevalence increases markedly with age (4) (5). In addition, it is the most common cause of hospitalisation in patients over 65 years of age (6). There has been an increase in the prevalence of HF in the past decades, at least partially because of the overall increase in the

number of elderly people due to the longer life expectancy, as well as due to advances in the treatment of precipitating factors for the development of HF, such as the acute coronary syndromes (7). Similar to the prevalence, the incidence of HF increases with age and has been shown to be higher in males compared with females in all age groups (8).

Apart from the data about the overall prevalence of HF, estimates of the prevalence of HF with preserved and reduced systolic function have been separately reported by studies, which assessed the LV systolic function. Among patients with symptomatic HF in population-based and hospital studies, approximately half of them have HF with systolic dysfunction and the other half have normal or near-normal systolic function (9) (10) (11). These studies also revealed differences in the characteristics of patients with HFpSF and HFrSF; patients of the former group are older, more likely to be female and have more frequently history of hypertension and diabetes. In contrast, patients of the latter group are younger and more likely to be male.

HF is associated with poor prognosis; data based on population-based studies prior to the modern era of treatment showed that within 5 years of diagnosis, approximately 60-70% died (12). Similarly, mortality and morbidity following hospitalisation with HF is markedly high. Recent data suggest that advances in the prevention and management of HF have resulted in decline in HF hospitalisation rates; however, the improvement in long-term mortality, although statistically significant, is clinically modest (13). Interestingly, various studies reported similar prognosis between HFpSF and HFrSF (14) (15). Nevertheless, a meta-analysis showed that patients with HFpSF had mortality approximately half that of patients with HFrSF (16).

1.1.3 Aetiology - Pathophysiology of HF

The aetiology and pathophysiology of HFrSF has been extensively investigated. The ventricular systolic dysfunction results from loss of a critical number of cardiomyocytes due to myocardial injury, or alternatively in response to a disruption of the myocardium ability to generate force, thereby altering the cardiac contractility (17). The predisposing factors may be of a sudden onset, as in the case of a myocardial infarction (MI); alternatively, they may have a gradual course with pressure or volume overloading, such as in hypertension or valvular heart disease. Moreover, hereditary factors related to genetic cardiomyopathies or exogenous factors with cardiotoxic effects can cause HF. Irrespective of the nature of the underlying aetiology, the damage to the myocytes and the extracellular matrix leads to changes in the morphology and structure of the ventricles (remodeling), resulting in deterioration in their systolic function (18) (19). The progression of the HF syndrome due to remodeling occurs in two main ways (20). Inter-current acute coronary events produce further damage and decline in the pumping capacity of the heart. Alternatively, compensatory mechanisms are activated due to the reduced systolic function with over-expression of biologically active molecules, which exert systemic effects. The above adaptive neurohumoral mechanisms, although beneficial for the maintenance of cardiovascular homeostasis in the short term, result in mechanical and electrical dysfunction of the heart in the long term; they also exert deleterious effects on other organs leading to a vicious cycle in which the heart is unable to provide adequate tissue perfusion with further progression of HF over time (Figure 1-1)

In contrast to HFrSF, the pathophysiology of HFpSF remains to be fully elucidated. Diastolic dysfunction, with impairment of LV relaxation and compliance, is likely to exert a principal role in these patients (21). Moreover, other systemic factors, as the vascular distensibility (22), might also play a pathophysiological role; however, the significance of activated neurohumoral pathways remains unclear in patients with HFpSF

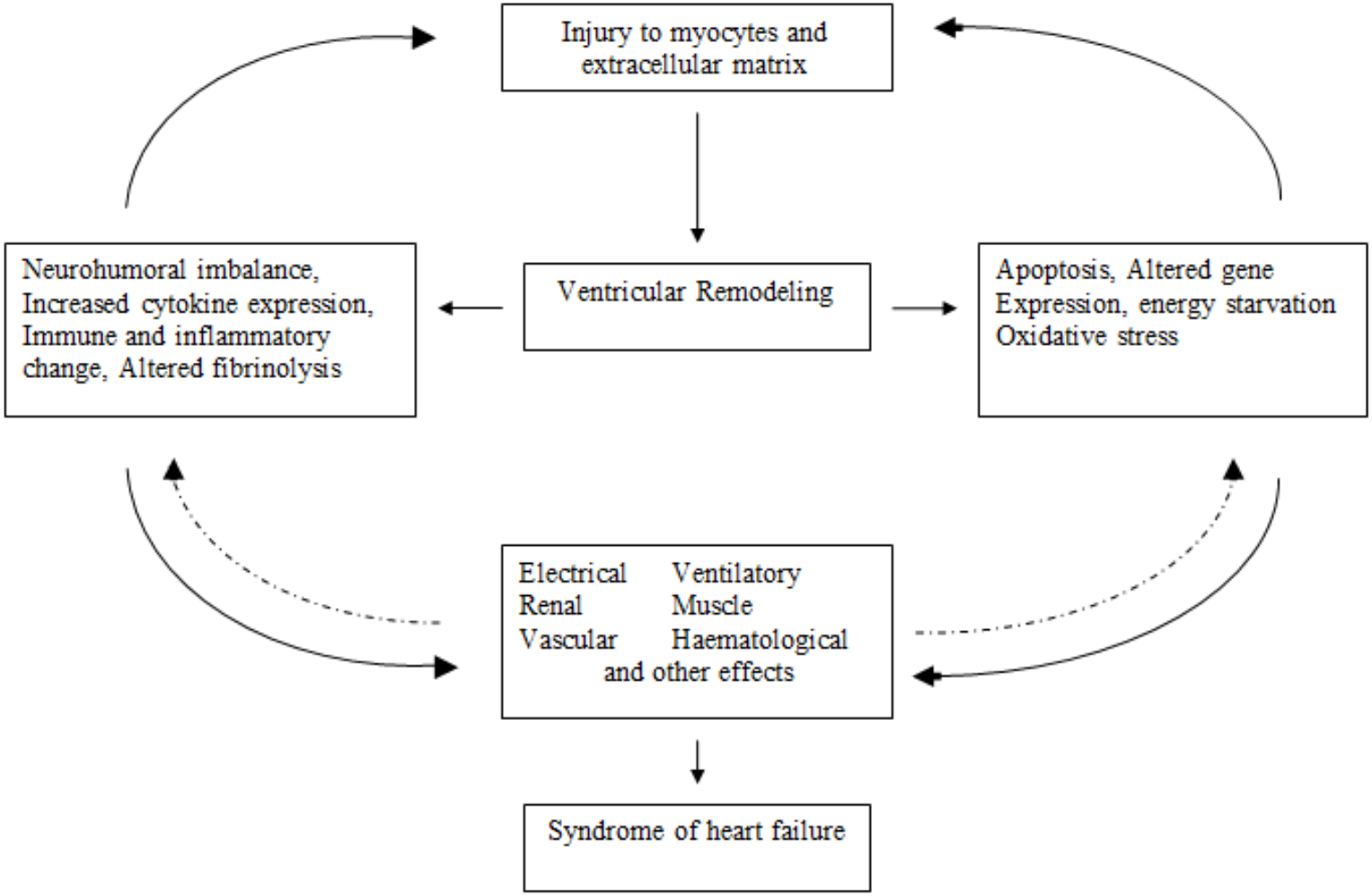


Figure 1-1. Pathophysiology of HF due to ventricular remodeling. Adapted from McMurray et al. (20)

1.1.3.1 Neurohumoral activation and the RAAS in HF

The neurohumoral adaptive responses that occur in HF due to LV systolic dysfunction (LVSD) include mainly the activation of the SNS (23) (24) and the RAAS (25) (26). In addition, other pathways related to the expression of cytokines and other inflammatory mediators are also up-regulated (27) (28). In parallel, counter-regulatory systems, such as natriuretic peptides, are stimulated antagonising the effects of the SNS and RAAS activation (29) (30).

The adrenergic nervous system is one of the first adaptive pathways that are activated early in the course of HF with systolic dysfunction. The SNS exerts positive cardiac inotropic and chronotropic effects in order to restore the decrease in the cardiac output (25). It also leads to peripheral vasoconstriction and stimulation of the nonosmotic release of arginine vasopressin, which causes antidiuresis and further enhances vasoconstriction, aiming to maintain the organ and tissue perfusion. Furthermore, the SNS is a stimulator of the RAAS, which in turn by secreting angiotensin II and aldosterone, results in further vasoconstriction and increase in intra-vascular volume.

The main mechanisms contributing to RAAS activation in HF include sympathetic adrenergic stimulation, decrease in intravascular volume and renal hypoperfusion, which promote renin release from a juxtaglomerular apparatus (JGA) in the kidneys (31). Renal perfusion pressure is principally involved in the regulation of renin secretion. Specific type of cells in the afferent arterioles sense changes in pressure and transmit signals to the juxtaglomerular apparatus, which in turn modifies the release of renin into the circulation. The JGA regulates also renin secretion through the macula densa cells, which are specific epithelial tubular cells at the renal distal tubule, lying in close proximity with the afferent arterioles. These cells sense Na^+ flux through the $Na^+K^+2Cl^-$ transporter and give constantly signals to the JGA to

adapt the renin secretion (32). In addition, the sympathetic system exerts a dual effect, with beta-adrenergic receptors stimulating and alpha-adrenergic receptors suppressing renin release (33). Apart from these regulatory factors there is also a negative feedback mediated by angiotensin II, which suppresses the release of renin by the juxtaglomerular cells. The clinical importance of this negative feedback circuit has been fully manifested with the use of RAAS inhibitors which increase renin levels several-fold (34).

The renin secreted by the JGA, acts on the circulating angiotensinogen, which is synthesised in the liver, to form the biologically inactive decapeptide angiotensin I. This is converted by angiotensin-converting enzyme (ACE) into the biologically active octapeptide angiotensin II. Angiotensin II effects are mediated through specific angiotensin II receptors on the cell membranes. Activation of angiotensin II type 1 (AT_1) receptors promotes various effects including systemic and renal vasoconstriction and enhances the activity of the SNS (35) (36) (37). Angiotensin II also stimulates via the same receptors the release of sodium-retaining hormone aldosterone from the adrenal cortex. The actions of angiotensin via the AT_1 receptor aim to restore the blood volume and renal perfusion and to maintain the circulatory homeostasis. However, the sustained activation of these receptors leads to adverse effects, including oxidative stress, vascular smooth muscle cell (VSMC) and cardiomyocyte hypertrophy with vascular and ventricular remodeling (38) (39) (40). In contrast, the effects of angiotensin II acting via angiotensin II type 2 (AT_2) receptors have been less well characterised, but evidence suggests that activation of AT_2 receptors attenuates some of the effects mediated by AT_1 receptor by inhibiting cell growth and promoting vasodilation (41) (42).

Apart from angiotensin II other peptides of the RAAS family have recently received increased attention in HF. Angiotensin 1-7 is a heptapeptide that is generated form

angiotensin I and angiotensin II. ACE2, a homologue of ACE, is expressed mainly in the heart, kidneys and vasculature and degrades angiotensin I to angiotensin 1-9 and angiotensin II to angiotensin 1-7 (43) (44). Angiotensin 1-9 is further degraded to angiotensin 1-7 by ACE (44). The expression of angiotensin 1-7 has been shown to be up-regulated in failing human heart ventricles (45). Similarly, the expression of ACE2 in the myocardium has been found to be increased in patients following MI and in patients with HF (45) (46). Angiotensin 1-7 is an active peptide and exerts its effects by binding to the receptor Mas (47). There is growing evidence that angiotensin 1-7 is part of an axis, which counterbalances some of the angiotensin II actions. ACE2 degrades angiotensin II to angiotensin 1-7, reducing the concentrations of the former and its vasoconstricting, proliferative and hypertrophic effects on myocardium. Moreover, angiotensin 1-7 prevents angiotensin II- induced cardiac hypertrophy and fibrosis independent of blood pressure (48) (49) (50). Furthermore, angiotensin 1-7 has also been shown to have vasodilatory properties in animals, however, that has not been replicated in healthy subjects or patients with HF (51) (52).

1.1.3.2 Aldosterone secretion in HF

The secretion of aldosterone, which is the main mineralocorticoid, is stimulated by RAAS activation in patients with HF. In healthy subjects, whose sodium intake is normal, aldosterone secretion ranges from 270 to 485 nmol per day; in patients with HF, aldosterone secretion may reach 1100 nmol per day (53)**.** The secretion of aldosterone in HF per se, however, is difficult to be evaluated as most of the available data come from studies with patients taking diuretic therapy or some form of a RAAS inhibitor (54) (55) (56)**.** Diuretics are well known to stimulate aldosterone synthesis, thus, raised concentrations of plasma aldosterone and renin in patients with HF may be due to the HF itself, to diuretic treatment or both. In contrast, ACE inhibitors or angiotensin receptor blockers (ARBs) suppress aldosterone secretion. A small number of early studies with untreated HF patients provided

evidence with regards to aldosterone secretion in patients not treated with a diuretic or a RAAS inhibitor. In patients with mild to moderate HF, plasma concentrations of aldosterone were well within the normal range (57)**.** In patients with untreated severe HF, aldosterone levels were not universally increased (58)**.** The wide variation of aldosterone levels in the above studies has been attributed to the degree of compensatory expansion of the circulating volume, which in turn suppresses renin and consequently aldosterone secretion (59). Indeed, plasma renin was not universally increased in patients with untreated severe congestive HF (58). Moreover, normal renin levels were reported in patients with untreated mild congestive HF (60)**.** In accordance, patients with untreated LVSD or HF had normal plasma renin activity (PRA) (61)**.** Overall, plasma renin and aldosterone levels in HF reflect a dynamic interplay between pathways with stimulating and inhibiting effects on aldosterone secretion. In addition to these regulatory systems, other factors, such as the reduced aldosterone clearance due to hepatic hypoperfusion or congestion, further contribute to the great variation of aldosterone levels in patients with HF (62) (63)**.**

The effect of aldosterone in the kidneys is to promote mainly sodium retention. In untreated patients with HF total body water content and extracellular volume are increased by more than 15% and 30% respectively (58)**.** In HF, there is evidence of prolonged sodium-retaining action of aldosterone in kidneys. In healthy subjects, administration of aldosterone results in an initial increase in extracellular fluid volume; however, the excretion of sodium in urine gradually increases despite the aldosterone sodium retaining effects (64). The exact mechanisms of the above phenomenon have not been clearly elucidated, but it is generally agreed that the increase of sodium delivery to the distal nephron, overrides the sodium retaining capacity of aldosterone and contributes to the re-establishment of sodium balance (64)**.** In patients with HF, the activation of the SNS and RAAS enhances sodium absorption in the proximal tubules with subsequent decrease in sodium delivery in the distal nephron

(65)**.** Under these conditions mineralocorticoids exert sodium retaining effects at their maximum. Thus, the overall sodium absorption throughout the nephron segments is almost complete, promoting persistent fluid retention in patients with HF.

Apart from the classic epithelial properties related to sodium and water retention, aldosterone has been increasingly recognised to exert other nonepithelial effects related to the cardiovascular system that contribute further to HF progression; these cardiovascular actions are described in detail in section 1.3. Moreover, aldosterone blockade has emerged as one of the principal treatment strategies in patients with HFrSF and the current evidence from clinical studies is described further in section 1.4.

1.1.3.3 Cortisol secretion in HF

Apart from the mineralocorticoid actions, there is growing evidence about potential detrimental effects that cortisol exerts on the cardiovascular system, indicating that glucocorticoids might play a distinctive role in HF pathophysiology (66) (67)**.** Cortisol, which is the main glucocorticoid in humans, is secreted like aldosterone by the adrenal glands; in humans the daily cortisol secretion is 41- 110 mmol, approximately 200 to 300 times higher than the daily aldosterone production (68)**.** In contrast to mineralocorticoids, the secretion of glucocorticoids has not been extensively studied in patients with HF. Plasma cortisol levels were found to be higher in patients with acute decompensated HF, in a series of early studies that included a very small number of patients (69) (70)**.** In patients with untreated severe HF, serum cortisol levels were within the normal range, albeit 2.5- fold higher compared with healthy control subjects (58)**.** Another study reported higher serum cortisol levels in patients with chronic HF and cachexia compared with HF patients without cachexia (71)**;** however, no difference in cortisol levels was elucidated between non-cachectic patients with HF and healthy subjects in this study. Moreover, in patients with

chronic HF morning cortisol levels were well within the normal range and no evidence of major activation of the HPA axis was present (72) (73)**.** In these patients, cortisol levels were not associated with markers of disease severity, cardiac cachexia or inflammation status. Interestingly, cortisol but not ACTH, which is the principal regulator of glucocorticoid synthesis, was independently associated with cardiovascular outcomes**.** These findings suggest that cortisol is not merely a nonspecific indicator of cardiac cachexia or stress, but might exert specific deleterious effects on the cardiovascular system. Thus, the secretion of glucocorticoids in patients with HF merits further investigation.

1.2 Synthesis and secretion of mineralo- and gluco- corticosteroids

1.2.1 Synthesis of aldosterone and cortisol (Figure 1-2)

The adrenal cortex consists of three zones. The outermost zone is the zona glomerulosa (ZG) where the cells are arranged in whorls. Zona fasciculata (ZF) lies just beneath ZG and zona reticularis (ZR), which is the innermost, surrounds the adrenal medulla. The cells in the last two regions are arranged in columns and produce glucocorticoids and sex steroids, while ZG is responsible for aldosterone biosynthesis. Unlike other hormones, the capacity of intracellular storage of corticosteroids is limited and the secretion rate is directly related to the activity of the steroidogenic pathways. The fundamental substrate for adrenal steroid synthesis is cholesterol. In human subjects, most of cholesterol used in the adrenal steroidogenic pathways is taken from circulation where it is mainly transported as low density lipoprotein (LDL) or high density lipoprotein (HDL) (74). In addition, de novo adrenal cholesterol synthesis may provide additional substrate for adrenal steroidogenesis but to a smaller extent. After intracellular deposition, cholesterol is transferred from the outer to the inner mitochondrial membrane, which is the rate-limiting step in steroidogenesis and is mainly mediated by the steroidogenic acute regulatory protein (StAR) (75)**.** Following this

step, cholesterol is subjected to a series of reactions to generate mineralo- and gluco-corticoids. Most of the enzymes that mediate the above reactions belong to the P450 cytochrome family of the haem-containing enzymes. In the inner mitochondrial membrane, the cytochrome P450 side chain cleavage enzyme, which is located in all three zones of adrenal cortex, performs successive hydroxylations of cholesterol to generate pregnenolone, which is transported to the cytosol. Subsequently, 17alpha-hydroxypregnenolone is synthesised from pregnenolone in the ZF/ZR by 17alpha-hydroxylase. Pregnenolone and 17alpha-hydroxypregnenolone are converted by 3beta-hydroxysteroid dehydrogenase/isomerase in the endoplasmic reticulum to progesterone and 17alpha-hydroxyprogesterone respectively, which are subsequently hydroxylated by 21-hydroxylase and converted to 11-deoxycorticosterone (DOC) and 11-deoxycortisol respectively. 21-hydroxylase is located in the smooth endoplasmic reticulum and expressed in all three zones of the adrenal cortex (76)**.**

The newly synthesized DOC in ZG enters the mineralocorticoid-producing pathway responsible for aldosterone generation. DOC is converted to aldosterone by aldosterone synthase, which catalyses the final enzymatic reactions in ZG. Aldosterone synthase, a cytochrome P450 enzyme, is located in the inner mitochondrial membrane of the ZG and catalyses three consecutive steps, converting DOC into corticosterone (11beta-hydroxylation), corticosterone to 18OH-corticosterone (18-hydroxylation) and finally 18OH-corticosterone to aldosterone (18-oxidation) (77)**.** These final steps are known as the 'late pathway' in aldosterone biosynthesis.

Similar to DOC with regards to aldosterone secretion in ZG, 11-deoxycortisol is the main substrate for cortisol production in ZF, the main glucocorticoid in humans; 11-deoxycortisol diffuses to the inner mitochondrial membrane, where is hydroxylated by 11beta-hydroxylase

to generate cortisol (78). 11beta-hydroxylase, apart from catalysing the production of cortisol, also mediates the conversion of DOC to corticosterone in ZF, similar to aldosterone synthase's enzymatic action in ZG. Nevertheless, 11beta-hydroxylase , which is principally controlled by ACTH, catalyses 18-hydroxylation poorly and in contrast with aldosterone synthase, which is mainly regulated by angiotensin II and potassium, does not catalyse 18-oxidation. As a consequence, aldosterone secretion is exclusively restricted in ZG (77)**.**

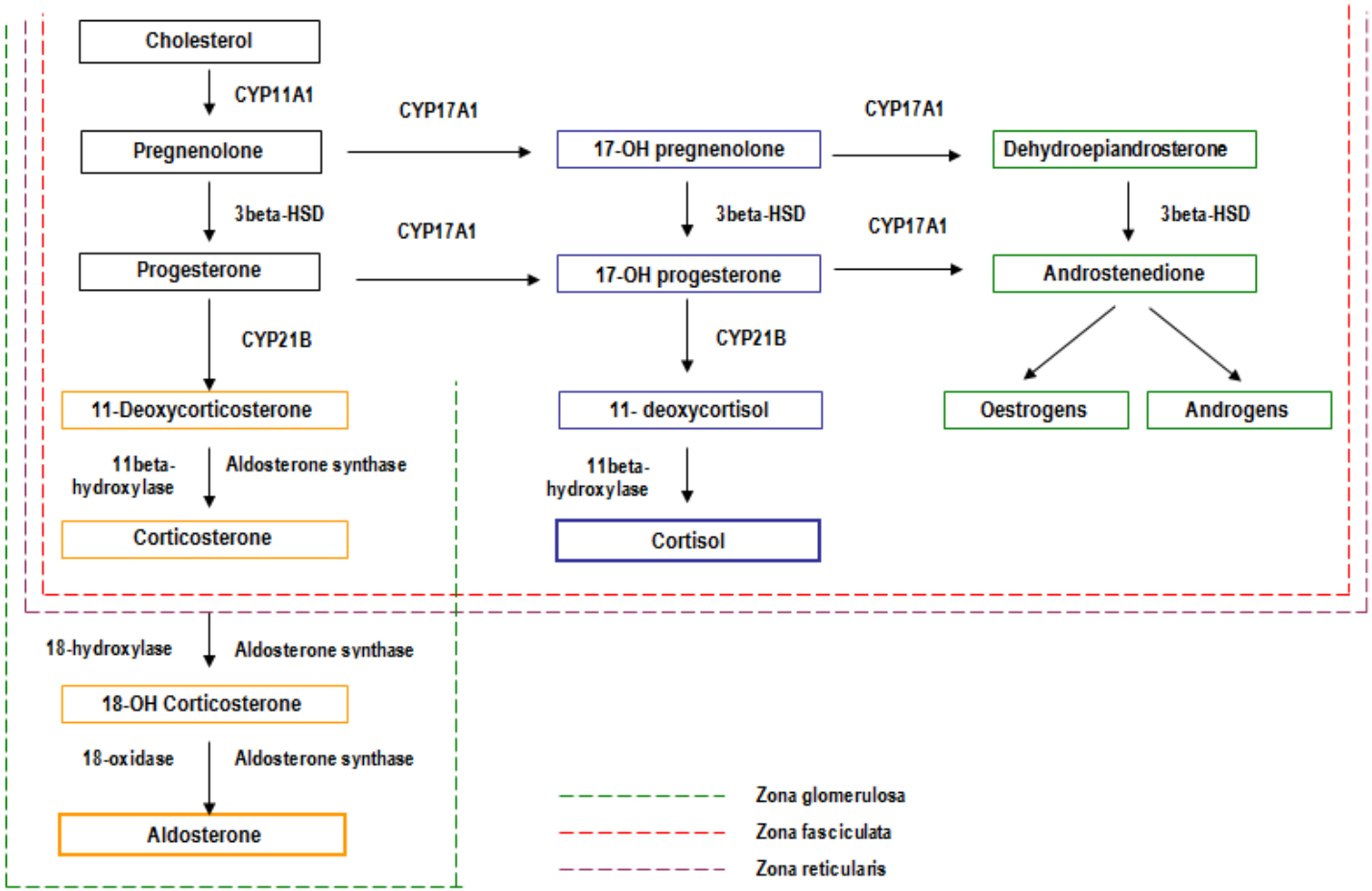


Figure 1-2. Adrenal corticosteroid biosynthesis

1.2.2 Metabolism of corticosteroids

The metabolism of corticosteroids is complex and mostly tissue dependent. The main cortisol metabolites are 5alpha-tetrahydrocortisol, 5beta-tetrahydrocortisol and cortisone (Figure 1-3). The 5-tetrahydro-compounds are formed in the liver and are conjugated with a glucuronide to form water-soluble compounds that are excreted by the kidneys. Cortisol, in addition, is converted by 11beta-hydroxysteroid dehydrogenase 2 (11beta-HSD2) to inactive cortisone in classic epithelial tissues (renal tubules, colon and salivary glands) (79). Cortisone, similarly to cortisol, is metabolised in the liver and excreted as tetrahydrocortisone in the urine.

11beta-HSD2 enzyme plays a critical role in the metabolism and action of corticosteroids. 11beta-HSD2 regulates the concentration of active glucocorticoids available to bind and activate the glucocorticoid receptors (GRs) by converting the active cortisol to biologically inactive cortisone (80). Additionally, by the same conversion, it mediates the aldosterone specificity for the mineralocorticoid receptors (MRs) in classic epithelial tissues (section 1.3.1). An isoenzyme, 11beta-hydroxysteroid dehydrogenase 1 (11beta-HSD1), is mainly found in liver and adipose tissue and preferentially converts cortisone to cortisol amplifying GR activation (81) (82).

Similar to glucocorticoids, aldosterone is metabolised in the liver to form mainly tetrahydro-aldosterone, which is mainly excreted in the urine as a glucuronide conjugate.

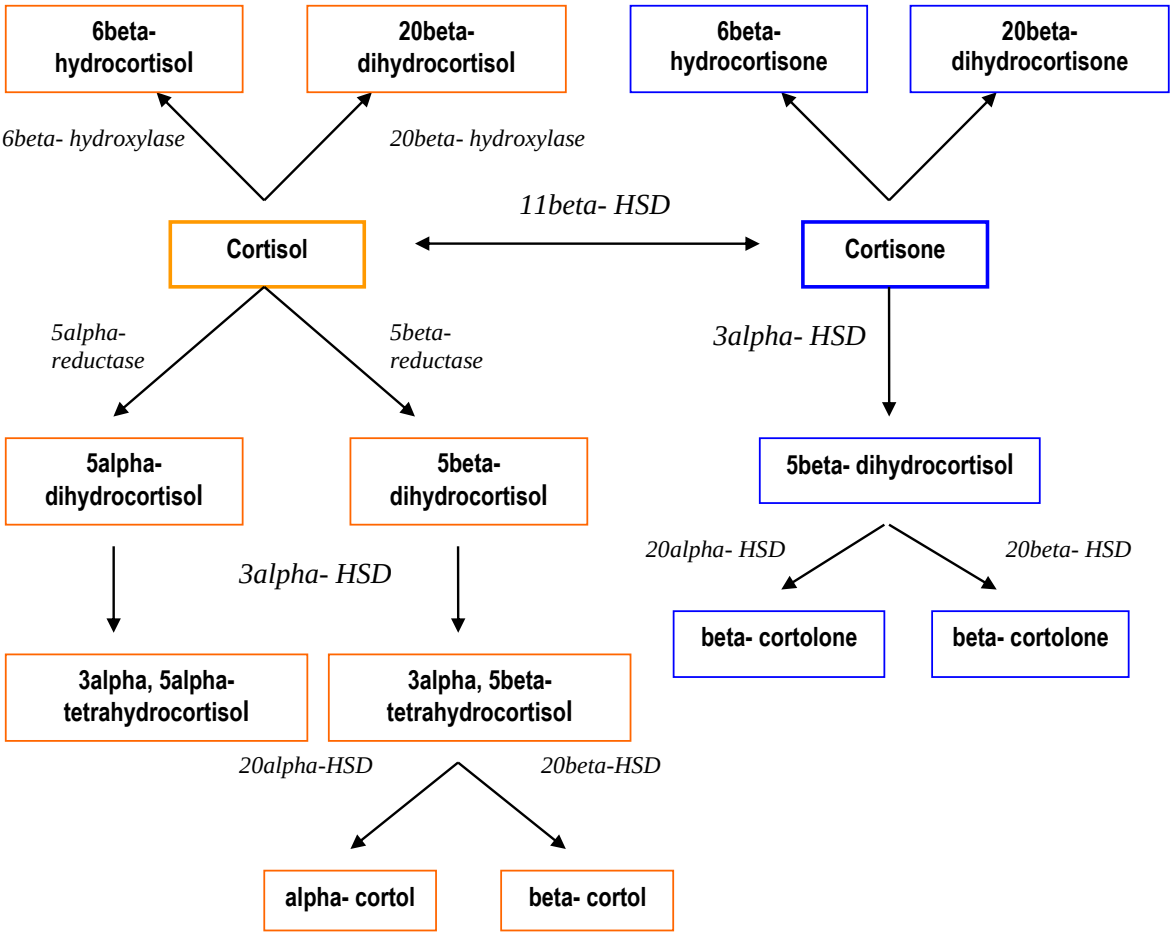


Figure 1-3. Metabolism of glucocorticoids

1.2.3 Regulation of aldosterone secretion

1.2.3.1 RAAS

Renin is one of the principal regulators of aldosterone secretion. It is released by the juxtaglomerular cells in response to a decrease in the intravascular volume, sympathetic stimulation and reduced sodium concentration at the macula densa. As previously described, renin acts on angiotensinogen secreted by the liver into circulation to produce angiotensin I, which is converted into angiotensin II by ACE that is located mainly in the lungs and vascular tissue. Angiotensin II, apart from the potent vasoconstricting properties, stimulates aldosterone secretion from the adrenal cortex through the AT_1 receptor. The expression of AT_1 receptors exhibits zonal distribution, contributing to the regulation of *CYP11B2* expression in ZG by angiotensin II (77). The ZG cells are very sensitive to angiotensin II, especially under circumstances of sodium-depletion (83) (84). Moreover, the response of these cells to angiotensin II is rapid, within minutes, indicating that aldosterone is immediately synthesised from intermediate compounds or de novo from cholesterol with no new protein synthesis (85). On the other hand, chronic stimulation by angiotensin II up-regulates *CYP11B2* expression and induces ZG cell proliferation and hypertrophy (86) (87). The increased aldosterone secretion resulting from angiotensin II stimulates in turn the expression of tissue ACE and AT_1 receptors (88) (89) (90). Thus, a positive feedback circuit is present in which angiotensin II binds to the AT_1 receptors in ZG and stimulates the secretion of aldosterone, which in turn leads to up-regulation in ACE expression and angiotensin II synthesis, with further increase in aldosterone levels. That represents a vicious cycle with a distinctive role in patients with HF, as it is likely to contribute to the raised aldosterone levels in these patients despite inhibition of RAAS (section 1.5.1).

Apart from angiotensin II the heptapeptide angiotensin III also stimulates aldosterone secretion. Angiotensin III is generated from angiotensin II by aminopeptidase A, a membrane

bound enzyme that is highly expressed in the kidney and especially in glomerular cells (endothelial, mesangial and podocytes) and the renal proximal tubule cells (91) (92). Aminopeptidase A cleaves the N-terminal amino acid from angiotensin II, which is converted to angiotensin III. Angiotensin III increases aldosterone secretion both in vivo and in vitro (93) (94) (95)**.** However, it is not fully elucidated whether angiotensin III exerts direct or indirect effects on aldosterone production or these effects are mediated by a specific receptor. It has been recently shown that aldosterone secreting effects of angiotensin III are partially mediated via the AT_2 receptor and that might contribute to aldosterone escape in patients with HF despite treatment with ARBs (96).

1.2.3.2 Potassium

Extracellular potassium is a major regulator of aldosterone secretion and potassium excess directly stimulates aldosterone secretion, independently of other regulatory mechanisms. The ZG cells are sensitive to changes in the extracellular potassium and respond even to small increments with aldosterone secretion (97) (98). Although potassium and angiotensin II exert independent effects on aldosterone production, a synergism between the two agonists has been identified resulting in enhancement of the individual trophin effects (99) (100). Thus, a fluctuation in potassium levels modifies the stimulating effect of angiotensin II on aldosterone production (101). However, it has been proposed that acute stimulation by angiotensin II impedes the potassium regulatory pathway, switching the adrenal to an angiotensin II-dependent model (77).

Potassium acutely stimulates aldosterone secretion by acting mainly in the early steps of the mineralocorticoid pathway (102). In addition, chronic potassium excess up-regulates the transcription of aldosterone synthase, stimulating the late pathway in aldosterone biosynthesis; increase in the dietary potassium results in up-regulation of aldosterone

synthase mRNA transcription in ZG (103) (104). Overall, potassium and aldosterone are part of a feedback circuit where increased potassium levels in the extracellular compartment stimulate aldosterone, which then increases kaliuresis and lowers potassium levels.

1.2.3.3 ACTH

ACTH is the principal endogenous stimulus to the main glucocorticoids, and the ZG is less dependent than the ZF upon ACTH control. Animal studies have shown that the aldosterone synthase expression in ZG, but not the 11beta-hydroxylase expression in ZF, is maintained despite low circulating levels of corticotrophin (105)**.** ACTH, however, plays an important role in the regulation of aldosterone secretion as a potent short-term secretagogue and increases aldosterone secretion in a dose-dependent manner (106). In contrast, during a continuous ACTH infusion at pharmacological doses, aldosterone levels return to normal in 24-72 hours, while cortisol levels remain elevated (107). In addition, long term continuous infusion of high exogenous ACTH doses results in hyperplasia and hypertrophy of ZF and is associated with a marked decrease in aldosterone secretion (108) (109)**.** The reduction of aldosterone secretion induced by ACTH is characterised by a sustained decrease in *CYP11B2* expression with suppression of the late steps in mineralocorticoid synthetic pathway (110) (111)**.** The molecular mechanisms for the morphological and functional responses of ZG to chronic ACTH stimulation are not clear but it is generally accepted that there is a transformation of adrenal ZG to ZF cell function (109) (110) (112)**.** Interestingly, the stimulation of ZG by an ACTH infusion is sustained if the infusion is pulsatile (113)**.**

Overall, apart from the short term effects, ACTH is considered to have a contributing role in the regulation of aldosterone synthesis in the long term. In humans with panhypopituitarism there was an impairment of aldosterone response to salt restriction and ACTH stimulation (114)**.** Moreover, aldosterone secretion exhibits a diurnal variation with higher levels in the

morning, indicating that an ACTH drive is at least partially involved in the regulation of mineralocorticoid synthesis (115).

1.2.3.4 Other factors stimulating aldosterone secretion

Apart from the three main aldosterone secretagogues, other substances have been reported to stimulate aldosterone secretion, variably and modestly compared to the main stimulators. Serotonin enhances mineralocorticoid synthesis in adrenal glomerulosa cells (116). In clinical studies, selective serotonin receptor agonists have been shown to stimulate aldosterone production in normal subjects (117). Other agonists include endothelin and vasopressin (118) (119). However, their exact role in the regulation of aldosterone secretion remains unclear.

Oxidized endogenous fatty acids have been reported to exert a direct stimulatory effect on aldosterone secretion; an oxidized derivative of linoleic acid, has been shown to have a potent aldosterone stimulating action on rat adrenal cells in vitro (120). In addition, plasma levels of oxidised derivatives of linoleic acid have been found to correlate positively with plasma aldosterone levels and body mass index (BMI) in humans (121). More recently, human adipocytes have been shown to secrete mineralocorticoid-releasing factors, which directly stimulate aldosterone production from adrenocortical cells in vitro and may represent a potential link between obesity and increased aldosterone levels (122). Thus, obesity and increased adipocity may promote aldosterone synthesis at least partially through adipose tissue-produced factors and endogenous fatty acids.

1.2.3.5 Factors attenuating aldosterone synthesis

Atrial natriuretic peptide (ANP) has been shown to suppress aldosterone secretion from the adrenal glands (123). ANP is secreted mainly from myocardial atrial cells in response to atrial distension and enhances vasodilation and diuresis (29) (124). Apart from the direct

effects on aldosterone levels, it also suppresses angiotensin II- and ACTH - induced aldosterone secretion in glomerulosa cells (125) (126). Moreover, it has been shown to inhibit the production of renin in juxtaglomerular cells reducing further angiotensin and aldosterone production (127) (128). The inhibiting effects on aldosterone secretion are not directly related to inhibition of steroidogenic enzymes and are mediated through intra-cellular signaling pathways that are activated through specific membrane receptors (129). The pathophysiological role of ANP becomes more prominent under circumstances of fluid overload, such as in patients with HF, where plasma natriuretic peptide levels are increased, exerting antagonistic effects to the actions of the RAAS mediators and the sympathetic nerve system.

Adrenomedullin (ADM) is another peptide with inhibitory effects on aldosterone secretion that was originally isolated from phaeochromocytoma tissues and has been found in different tissues, such as the adrenal medulla and endothelial cells (130) (131). ADM has been shown to inhibit angiotensin II- and potassium- induced aldosterone secretion in animal and human adrenocortical cells (132) (133). In animal studies, ADM has been found to attenuate aldosterone secretion after a few days of sodium-repletion (134)**.** In keeping with these findings, ADM has been shown to antagonise angiotensin II-induced but not basal aldosterone production in healthy subjects (135)**.** Although the role of ADM with regards to aldosterone secretion has not been fully characterised, findings from the above studies indicate that ADM probably exerts an inhibitory effect on mineralocorticoid secretion under conditions characterised by RAAS activation.

Other factors such as dopamine and somatostatin have also been shown to exert inhibiting effects on aldosterone secretion, however not as drastically as ANP and ADM (108).

1.2.4 Regulation of Cortisol secretion

ACTH is synthesised in the pituitary gland as part of a large precursor called proopiomelanocortin (POMC). ACTH synthesis requires proteolytic processing of POMC, which contains the sequences for other hormonal peptides, including the beta-endorphin and melanocyte-stimulating hormones (136)**.** ACTH is released into the circulation and stimulates secretion of glucocorticoids in the adrenal cortex.

The secretion of ACTH and therefore of cortisol is regulated by hormonal interactions within the HPA axis and by other stimuli that affect that circuit. The main secretagogue for ACTH secretion is the corticotropin releasing hormone (CRH), which is also synthesised as part of a larger precursor by neurons in the hypothalamus and is secreted into the pituitary portal blood (137). CRH increases the expression of POMC gene with subsequent increase in POMC and ACTH secretion by the hypophysis (138)**.** CRH secretion and consequently plasma ACTH and cortisol levels can be influenced by neural stimuli in response to stress (139)**.** While CRH is the major hypothalamic releasing factor for ACTH, other hormones such as vasopressin have the potential to stimulate ACTH release or to augment CRH-induced ACTH secretion, although at lower potencies (140). Additionally, other stimuli such as hypoglycaemia or fever stimulate CRH and ACTH levels and significantly contribute to the regulation of cortisol secretion (141) (142).

Under normal conditions there is a negative feedback loop between cortisol, ACTH and CRH. Cortisol decreases hypothalamic transcription of the POMC gene (143), inhibiting consequently ACTH release into the circulation. Glucocorticoids also suppress the transcription of CRH receptor gene (144) and might also exert a negative feedback at higher brain centers to attenuate the neural inputs to the hypothalamus.

In addition to the above mechanisms, there is a circadian rhythm in ACTH and cortisol secretion; their release shows pulsatile rhythmicity, with bursts of different amplitude throughout a day. The pulses occur with a frequency of 1 per 60-90 minutes (145) and increase in amplitude in the early morning hours reaching a peak prior to waking (146)**.** Subsequently, there is a decrease over the course of the day with minimum amplitude at midnight. This diurnal pattern occurs despite the negative feedback effects of cortisol on the hypothalamus and pituitary under normal circumstances.

1.3 Mineralo- and gluco-corticoid actions and cardiovascular system

1.3.1 Epithelial actions of aldosterone

Aldosterone exerts its classical effects on epithelial cells in the kidneys, colon and salivary glands, where it increases sodium and water reabsorption. In parallel, it increases potassium and hydrogen excretion contributing to electrolyte homeostasis. Aldosterone-responsive sodium transport within the kidneys is primarily mediated by the amiloride-sensitive epithelial sodium channel (ENaC) at the apical membrane of the distal convoluted and cortical collecting tubules (147)**.** Aldosterone induces up-regulation of ENaC activity through an increase in the number of channels in the apical membrane or alternatively by an increase in their open-probability (148) (149). It additionally increases the efflux of sodium from the epithelial cells to the intravascular compartment by a sodium-potassium ATPase (Na^+- K^+-ATPase) located in the basolateral epithelial membrane (140) (150). This is an energy-dependent process and generates a negative potential difference, which is counterbalanced with a concurrent reabsorption of Cl^- via the thiazide-sensitive Na^+/Cl^- co-transporter and K^+/H^+ secretion into the lumen (151)**.** Apart from the above effects to the sodium flux,

aldosterone up-regulates H^+-ATPase activity in the collecting duct and affects acid-base homeostasis (152).

Overall, the effect of aldosterone in the renal tubule is to promote sodium retention with concomitant hydrogen and potassium excretion in urine. The above effects are important not only under normal conditions but also in syndromes associated with activation of aldosterone secretion. In HF, the activation of RAAS results in aldosterone stimulation with concomitant sodium and fluid retention and expansion of the extracellular compartment. On the other hand, aldosterone blockade has been associated with hyperkalaemia in patients with HF (153).

1.3.1.1 Genomic actions

The classical aldosterone epithelial effects on the kidneys, colon and salivary glands are mediated by the MR, a member of the steroid/ thyroid/ retinoid nuclear receptor family (154). The MRs bind the main glucocorticoids (cortisol for man and corticosterone for rodents) and aldosterone with equal affinity (155). Glucocorticoids circulate in plasma in 100- to 1000-fold higher concentrations than plasma aldosterone levels and thus the MRs would predominantly be occupied by glucocorticoids (67). The selectivity of MR for aldosterone at epithelial tissues, however, is mediated by 11beta-HSD2, which converts cortisol to cortisone and corticosterone to dehydro-corticosterone, which exert no affinity for the MR (156). In this way the MRs at collecting ducts and other aldosterone epithelial target tissues are activated by aldosterone.

The MRs are located in the cytosol and are kept transcriptionally inactive in the absence of a ligand. Once activated by an agonist, the complex translocates to the nucleus where it binds to mineralocorticoid - responsive genes (157). The above interaction results in the induction

of a number of aldosterone-induced proteins (AIPs), which exert a wide range of actions on apical membrane and basolateral Na^+- K^+-ATPase activity in epithelial cells.

One of the best characterized AIPs is the serum glucocorticoid-regulated kinase 1 (SGK1). Aldosterone up-regulates the expression of SGK1 at the distal tubule of the kidneys and in the colon (158) (159). SGK1 in turn activates ENaC partially by up-regulating the expression of ENaC gene subunits (160). However, it has been recognised that SGK1 action on ENaC activity occurs mainly through an interaction with a regulatory protein also known as neuronal precursor cells-expressed developmentally down-regulated protein 4-2 (Nedd4-2), which mediates the ENaC turnover (161). This protein ligase interferes with the ENaC subunits at the apical membrane, promoting channel internalisation and degradation. SGK1 impairs Nedd4-2 interaction with ENaC and therefore increases the ENaC density in the distal nephrons (162) (Figure 1-4). Apart from the above actions on ENaC activity, SGK1 has also been shown to up-regulate the density of the inward rectifier K^+ channel, contributing to the kaliuretic effect of aldosterone (163).

Other proteins related to aldosterone action in transporting epithelia include the Kirstten Ras GTP-binding protein 2a, the glucocorticoid induced leucine zipper protein (GILZ) and the corticosteroid hormone-induced factor (CHIF) (85)**.** The precise actions of the proteins remain to be fully elucidated.

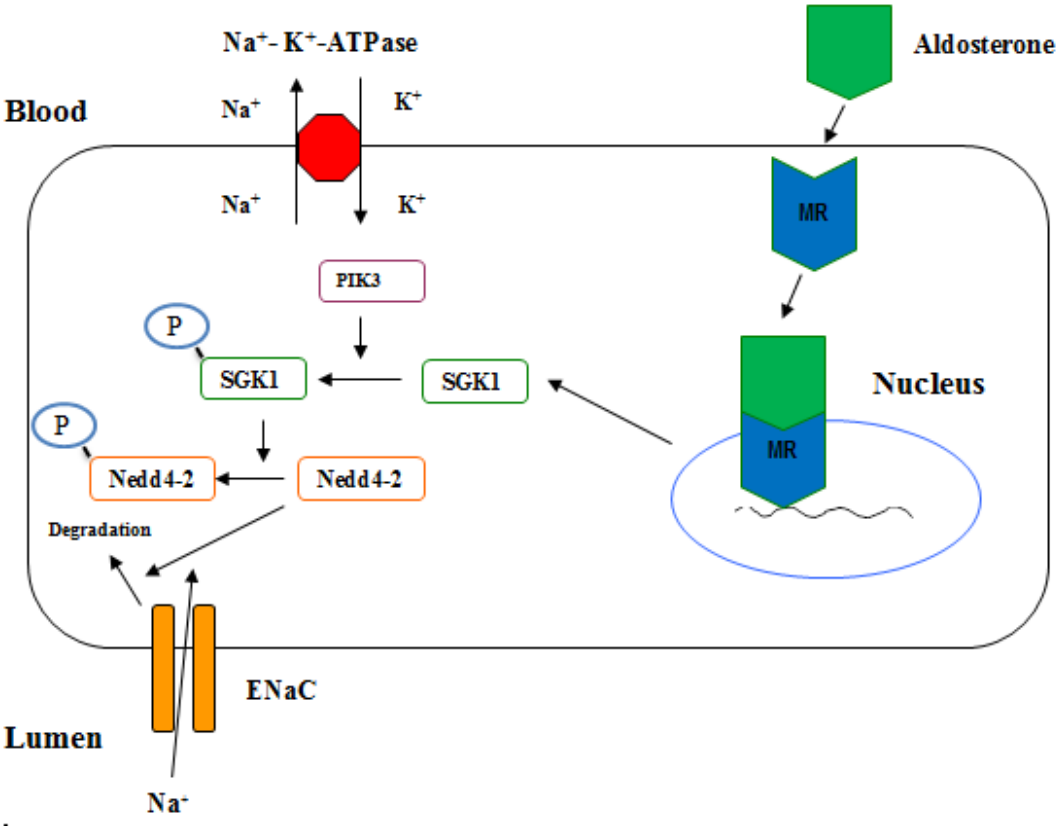


Figure 1-4. Genomic actions of aldosterone on epithelial tissues. Aldosterone binds to the mineralocorticoid receptor (MR) and the complex translocates in the nucleus where it increases the transcription of mineralocorticoid-responsive genes, resulting in the production of aldosterone -induced proteins. The activation of aldosterone-induced serum glucocorticoid-regulated kinase 1 (SGK1) requires phosphorylation by the phosphatidylinositol-3 kinase (PI3K). SGK1 impairs via phosphorylation the activity of the neuronal precursor cells-expressed developmentally down-regulated protein 4-2 (Nedd4-2) and the Nedd4-2- induced degradation of ENaC increasing the number of ENaC channels in the apical membrane.

1.3.2 Non genomic actions of aldosterone

In addition to the classic aldosterone effects related to the interaction of the hormone/ receptor complex with the deoxyribonucleic acid (DNA) regulatory elements, aldosterone has been shown to exert rapid nongenomic effects. These actions are independent of transcription or translation, and thus are not prevented by agents inhibiting the above processes (164) (165). Moreover, they appear within 5-10 minutes, a time course that precludes any effects on protein synthesis (166). The nongenomic effects have been described in a wide range of epithelial and nonepithelial cells including kidney tubule cells, skeletal and VSMCs and cardiac myocytes. Aldosterone rapidly affects the Na^+-K^+ pump activity in rabbit ventricular myocytes and exerts a negative inotropic effect in human atrial and ventricular trabeculae (167)**.** Other studies demonstrated a rapid increase in intracellular pH via the Na^+-H^+ exchanger in human arteries (168) and in kidney distal tubule cells induced by aldosterone (169). They also showed and a rapid rise of intracellular calcium in human mononuclear leucocytes and skeletal muscle cells in response to aldosterone (170) (171)**.** In healthy subjects aldosterone infusion induced rapid reductions in forearm blood flow (172) (173), although these findings have not always been replicated (174). In addition, aldosterone blunted the baroreflex response in healthy individuals (175) and decreased the heart rate variability in HF patients (176). Overall, the above effects are rather modest and may act by sensitising physiological responses to other synergistic stimuli, representing a cardiovascular fine-tuning system (177)**.**

Similarly to the genomic MR effects, some of the nongenomic effects are prevented by a classical MR blockers, indicating that the MR contributes partially to aldosterone-induced non-transcriptional effects (178); However, several of aldosterone-induced rapid effects are not attenuated by classical MR blockers; spironolactone had no effect on aldosterone's negative inotropic effect in human ventricular trabeculae (167)**.** Moreover, spironolactone did

not prevent the deterioration of myocardial contractile function due to coronary vasoconstriction following aldosterone infusion in canine hearts (179)**.** It has been hypothesised that some effects may be mediated by the classic MR in a non-transcriptional pattern and some others by a specific receptor**;** however, attempts to isolate a specific aldosterone receptor have been unsuccessful. Interestingly, there is evidence from other steroid pathways related to progesterone and oestrogens, that nongenomic effects are mediated via modulation of G-protein-coupled receptors (180). Similar mechanisms may occur for aldosterone (181)**.**

1.3.3 Non-epithelial actions of aldosterone

MRs have also been isolated in cells of non-epithelial tissues, including the cardiomyocytes, VSMCs and endothelial cells, circulating monocytes and the hippocampus (182). In the myocardium and hippocampus the expression of 11beta-HSD2 is extremely low or absent (183) (184) (185)**.** Thus, the MRs are mainly occupied by endogenous glucocorticoids in a tonic inhibition fashion, as the glucocorticoid-MR complex is not active under normal circumstances (186). In contrast, the VSMCs and endothelial cells express MRs and 11beta-HSD2, facilitating aldosterone specificity for the MR (187) (188). There is increasing evidence that aldosterone exerts deleterious non-epithelial effects on endothelial and vascular function, promotes myocardial fibrosis and has additional pro-arrhythmic effects (Figure 1-5).

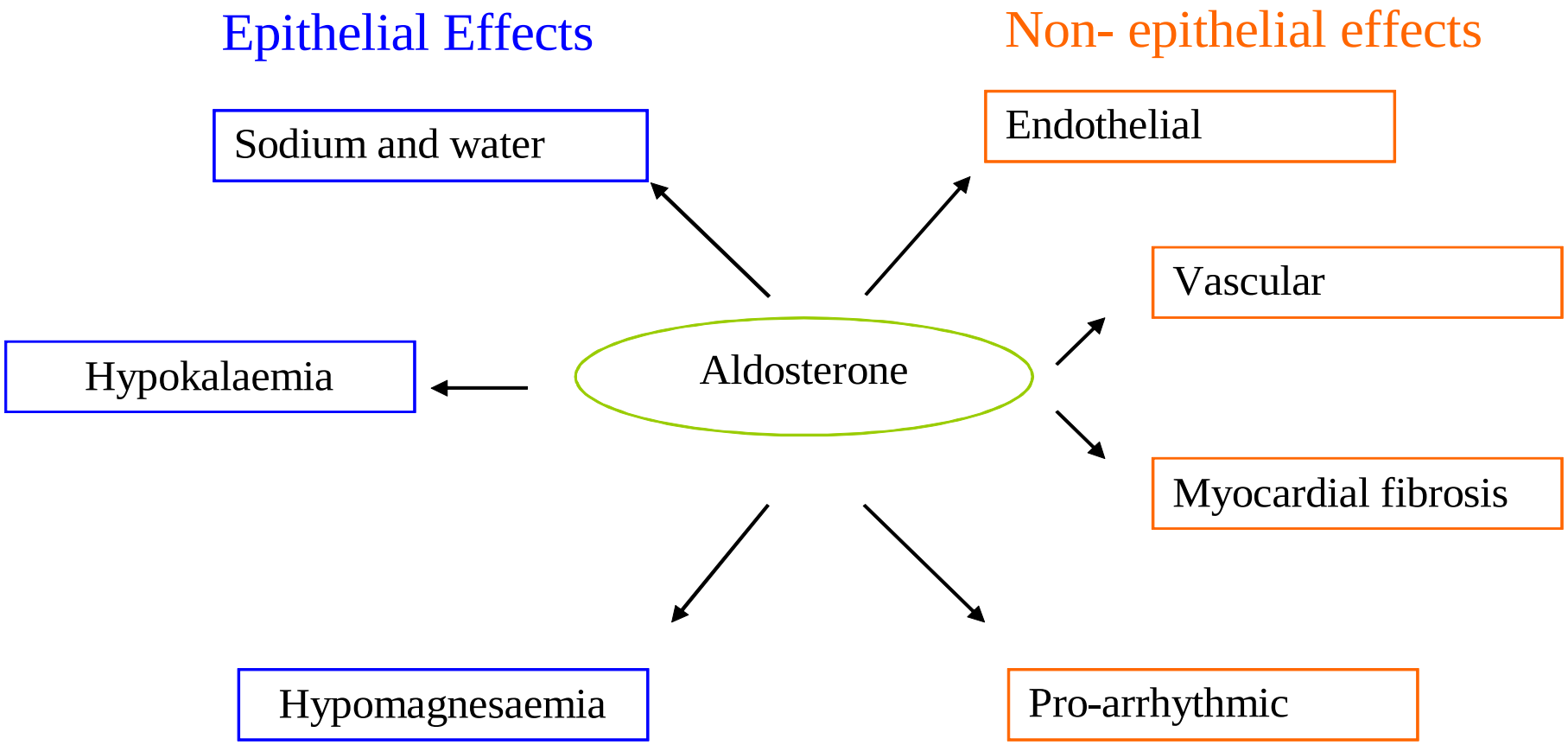


Figure 1-5. Aldosterone effects and cardiovascular system

1.3.3.1 Vascular Inflammation

Aldosterone has been shown to activate nicotinamide adenine dinucleotide phosphate (NADPH) oxidases in vascular smooth muscle and endothelial cells and to produce reactive oxygen species (ROS) (189) (190). Although ROS may derive from different pathways (mitochondria, xanthine oxidase, cyclooxygenase, or peroxidases), NADPH oxidases are the main stimulators of ROS generation in vascular tissues (191) (192)**.** The NADPH oxidase system comprises cytosolic and membrane-bound subunits that form an enzyme complex capable of producing superoxide anion (O_2^-) and other ROS; mineralocorticoids stimulate the transcription of various NADPH subunits, inducing up-regulation in ROS production (193). The ROS stimulate in turn the expression of pro-inflammatory factors, such the nuclear factor-kappa B (NF-kappa B) (194) (195), which in turn induce the generation of various adhesion molecules, chemokines and inflammatory cytokines (196) (197).

The oxidative stress and pro-inflammatory phenotype induced by mineralocorticoids in the vascular wall, appears to evolve to vascular inflammation under the synergistic effect of high salt diet (198) (199). Vascular and peri-vascular inflammation in the myocardium, with leukocyte infiltration, vasculitis and myocardial necrosis, was a common finding in animal models treated with aldosterone infusions and high salt diet. The aldosterone/salt combination in these studies increased the myocardial expression of various inflammatory markers, including osteopontin, intercellular adhesion molecule-1 and macrophage chemoattractant protein-1, which are not normally expressed in the heart. Similarly, in uninephrectomised rats that maintained on a high salt diet, peri-vascular macrophage infiltration and up-regulation of inflammatory cytokines were present following injections of subcutaneous mineralocorticoids (200). In all these studies, high salt intake was essential for aldosterone to exert its inflammatory effects on the vascular wall. The precise mechanisms by which high salt enables the inflammatory effects of aldosterone remain unclear. Activation of

MR causes intracellular calcium (Ca^{2+}) loading and a fall in cytosolic free-ionised magnesium (Mg^{2+}) in monocytes in the presence of high sodium (Na^{+}) (201). It has been suggested that the oxidative stress and a pro-inflammatory phenotype in mononuclear cells induced by intracellular calcium loading in the monocyte/macrophage system act as a substrate for aldosterone's pre-inflammatory action (202).

The vascular inflammation induced by aldosterone is reversed by MR blockade; spironolactone attenuated the effects of aldosterone on vascular oxidative stress in hypertensive rats (203). In addition, in heritable hyperlipidaemic rabbits, MR blockade resulted in down-regulation of ROS with reduction in free radical injury (204). Moreover, in a rat model with chronic pressure overload, treatment with eplerenone exerted beneficial effects on myocardial oxidative stress, suppressing the expression of the intercellular adhesion molecule-1 with concomitant reduction in macrophage infiltration and inflammation (205).

In patients with diabetic nephropathy blood pressure was reduced in response to spironolactone or amlodipine. However, only spironolactone reduced the urinary levels of the inflammatory markers, suggesting that aldosterone blockade attenuated vascular inflammation irrespective of blood pressure (206). In another study, hypertensive patients were randomised to receive spironolactone or chlorothalidone on top of treatment with a calcium-channel blocker and ARB (207). A significant decrease in blood pressure was present in both treatment arms but only spironolactone reduced the high sensitivity C-reactive protein (CRP). However, aldosterone blockade had no effect on CRP levels in a cohort of patients with chronic HF (208).

1.3.3.2 Endothelial dysfunction

Endothelial dysfunction is the end result of different pathophysiological pathways. Experimental and clinical studies have shown that decrease in bioavailability of endothelial nitric oxide (NO) results in impairment of endothelium-dependent relaxation (209) (210). Aldosterone induces down-regulation of endothelial NO availability in vitro and also affects endothelium-dependent vasoregulatory mechanisms by increasing ROS and oxidative stress in vivo (211). The generation of ROS leads to the oxidation of the NO synthase (NOS) co-factor tetrahydrobiopterin (BH4) and in the absence of BH4, endothelial NOS produces superoxide and hydrogen peroxide instead of NO, a process known as uncoupling (212) (213). This action of aldosterone on NO availability is similar to that of angiotensin II and there is evidence that a cross-talk occurs between the two RAAS mediators with regards to the endothelial NOS uncoupling (203). In addition, aldosterone has been shown to down-regulate the endothelial expression of glucose-6-phosphate dehydrogenase (G6PD), which mediates via the pentose phosphate pathway the generation of NADPH (214). The latter contributes to the maintenance of reduced glutathione intra-cellular stores, which participate in the neutralisation of ROS (215). Thus, the aldosterone-induced decrease in antioxidant reserve in endothelial cells via the above pathway contributes futher to the decrease in NO biovailability under conditions of increased generation of ROS. Moreover, aldosterone impairs the endothelial function by increasing the volume and stiffness of endothelial cells, inducing intercellular gaps in vitro (216).

The effects of mineralocorticoids on oxidative stress and endothelial function are attenuated by MR blockade. In a coronary ligation MI rat model, eplerenone normalised the production of ROS in the aorta and improved the endothelial function post-MI (217). In an experimental HF rat model, treatment with spironolactone on top of an ACE inhibitor reduced superoxide formation and up-regulated the endothelial NOS expression (218). In clinical studies, MR

blockade improved endothelium-dependent vasodilation in hypertensive patients with hyperaldosteronism (219). Furthermore, in patients with HF, treatment with spironolactone on top of an ACE inhibitor increased NO bioactivity and improved the endothelial vasodilator function (220) (221).

1.3.3.3 Myocardial Fibrosis

Treatment of uninephrectomised rats with a combination of aldosterone and salt induced accumulation of collagen with associated interstitial and perivascular myocardial fibrosis (222). The above effects were only seen in rats maintained on high and not on low salt diet. Interestingly, collagen accumulation in the aldosterone/ salt treated rats affected both the left and right heart, indicating that cardiac fibrosis was humoral rather than a haemodynamic effect of mineralocorticoid/ salt treatment. In keeping with these findings, in animal models co-infused with aldosterone peripherally and intra-cerebroventricular MR antagonist centrally, in order to maintain the blood pressure at normotensive levels, the degree of cardiac fibrosis was similar to that induced by aldosterone infusion alone (223)**.**

Aldosterone-induced cardiac fibrosis may be due to direct effects on the heart fibroblasts. Indeed, aldosterone has been shown to stimulate collagen synthesis by cardiac fibroblasts in vitro (224) (225); the above findings, however, were not always replicated (226) (227). In addition, other studies showed that aldosterone increases cardiac fibroblast proliferation instead (228). Apart from the direct effects on cardiac fibroblasts, it has also been shown that vascular and perivascular inflammation precedes myocardial fibrosis in animal models treated with aldosterone and salt; specifically, the primary inflammatory cells involved in peri-vascular inflammation and myocardial necrosis were monocytes and macrophages (200) (229). Recent studies have further focused on the specific role of MR activation of the monocyte/macrophage system on inflammation and cardiac fibrosis using

monocyte/macrophage-specific MR knockout mice (230)**.** Wild-type mice developed vascular macrophage infiltration with concomitant inflammation and cardiac fibrosis in response to mineralocorticoid/salt treatment. MR-knockout mice showed macrophage infiltration in the myocardial tissue but did not develop inflammation or fibrosis in response to mineralocorticoid/salt combination, indicating that macrophage MR activation is necessary for myocardial fibrosis but not for macrophage chemoattraction (Figure 1-6). On the other hand, endothelial MR activation up-regulates the expression of intracellular adhesion molecules, resulting in macrophages attaching to the endothelium (231). Overall, it appears that MR activation in endothelium and monocyte/macrophage system is responsible for both chemoattracting the macrophages to the endothelium and controlling their activities on arrival at the vascular and perivascular space, promoting inflammation and collagen disposition. In keeping with these considerations is also the finding that MR overexpression in cardiomyocytes did not result in myocardial fibrosis (232)**.** Moreover, when various components of inflammatory signaling pathways were targeted following aldosterone/salt treatment in animal models the collagen accumulation was reversed, indicating that myocardial fibrosis is at least partially a consequence of vascular and peri-vascular inflammation induced by mineralocorticoids (233) (234) (235) (236).

The antifibrotic effects of aldosterone blockers have been examined in both animal and clinical studies. MR antagonism prevented aortic inflammation and fibrosis in spontaneously hypertensive rats independent of blood pressure reduction (237). In a rat model of MI, aldosterone blockade reduced the reactive fibrosis in the viable myocardium without affecting the replacement collagen deposition in the infracted region (238). In rats with MI complicated with LVSD, eplerenone reduced collagen type I mRNA expression and collagen accumulation in the non-infarcted myocardium (239). In patients with HFrSF, spironolactone on top of ACE inhibitor therapy reduced plasma collagen turnover markers such as pro-

collagen type I carboxy-terminal peptide (PCIP), pro-collagen type I (PINP) and III (PIIINP) amino-terminal peptides (176) (240) (241). In addition, the impact of aldosterone blockade on myocardial fibrosis has additionally been investigated in patients with HFpSF. Eplerenone, on top of an ACE inhibitor or ARB and beta-blocker prevented the increase in PIIINP after one year in these patients (242).

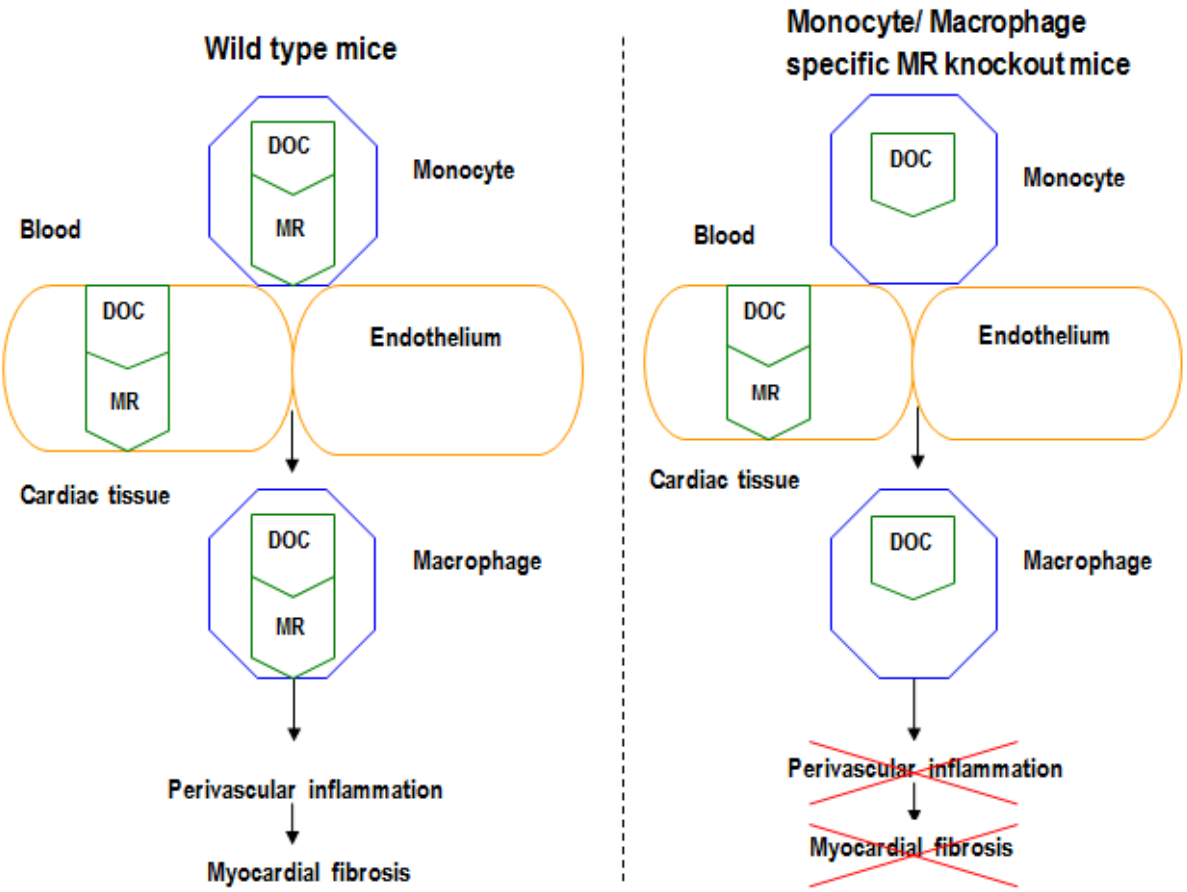


Figure 1-6. Specific deletion of the mineralocorticoid receptor (MR) in monocytes/ macrophages prevents perivascular inflamation and myocardial fibrosis but not adhesion of monocytes to the endothelium or infliltration of macrophages to the intramyocardial perivascular space in response to 11-deoxycorticosterone (DOC) and salt. Adapted from Dorrance (243).

1.3.3.4 Pro-arrhythmic effects

The myocardial collagen formation with fibrosis and interstitial remodeling induced by aldosterone is associated with electrical inhomogeneity and could potentially provide the substrate for potential arrhythmogenesis (244). However, there is growing, evidence that MR activation in cardiomyocytes exerts direct actions on their electrical properties increasing the risk of arrhythmias. Incubation of rat ventricular myocardial cells with aldosterone resulted in an up-regulation of sarcolemmal inward L-type calcium current (I_{ca}) expression in vitro**,** which induced in turn a decrease in transient outward potassium current (I_{to}) in ventricular myocytes (245) (246).The increase in I_{ca} density induced by aldosterone was attenuated by treatment with spironolactone, indicating a potential role of cardiomyocyte MR in the modulation of calcium cardiac ion currents (247). The effects of aldosterone on sarcolemmal calcium and potassium ionic currents prolong the ventricular action potential, providing the substrate for arrhythmias and increasing the risk for arrhythmic death (244)**.**

Consistent with the in vitro findings are also reports from in vivo studies. In a transgenic mouse model, conditional cardiomyocyte-specific MR overexpression resulted in ion channel remodeling with up-regulation of I_{ca}, down-regulation of I_{to} and action potential duration prolongation (232)**.** The electrical remodeling induced by cardiac MR over-expression was prevented by treatment with spironolactone, which reduced the high rate of ventricular arrhythmias observed in the above mouse model. Correspondingly, in a MI rat model, electrical remodeling was present early post-infarction, characterised by increased I_{ca} and decreased the I_{to} expression with concomitant prolongation of action potential duration; MR blockade prevented the electrical remodeling post-MI in that model (248)**.**

In clinical studies, aldosterone infusion resulted in impairment of baroreflex response in healthy subjects (175)**.** In patients with HF, MR blockade improved parasympathetic activity, heart rate variability and QT dispersion (176) (249) and increased furthermore the

cardiac neuronal uptake of norepinephrine, resulting in a decrease in ventricular arrhythmias (250)**.** The overall benefit of MR blockade on arrhythmic death was shown in the Eplerenone Post Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS), in which eplerenone, a selective MR blocker, reduced the risk of sudden cardiac death and all-cause mortality in 30 days after initiation of treatment in patients with HF and LVSD following MI (251) (section 1.5.3).

1.3.4 Main effects of glucocorticoids

Glucocorticoids exert their main actions by stimulation of the GRs; they bind to the GRs after entering into the cytoplasm, promoting dissociation of the GR from key heart shock proteins and translocation of the ligand – receptor complex to the nucleus. The binding of the complex to specific DNA sites results in the transcriptional activation of glucocorticoid-responsive genes (252). Apart from the GRs, cortisol binds to the MRs (155). However, as previously mentioned, in some but not in all tissues cortisol is converted to inactive cortisone by 11beta-HSD2 (253). Thus, the glucocorticoid effects depend upon whether the target tissues express GRs, MRs or 11beta-HSD2 (254).

Glucocorticoids exert their main biological effects on carbohydrate and protein metabolism as well as on the inflammatory and immune processes. In addition, glucocorticoids have weak mineralocorticoid activity under normal physiological conditions (255). It has been increasingly recognised that glucocorticoids exert detrimental effects on the cardiovascular system.

1.3.5 Cardiovascular effects of glucocorticoids

1.3.5.1 Endothelial dysfunction

Glucocorticoids have been shown to affect the endothelial NO system. Hydrocortisone and dexamethasone inhibited the expression of inducible NO synthase in vascular endothelial cells via a GR-mediated mechanism in vitro (256)**.** Dexamethasone decreased the availability of NO synthase cofactor tetrahydrobiopterin, promoting the synthesis of ROS in vitro (257)**.** Moreover, in animal studies, endothelial NO synthase expression was down-regulated in the aorta of dexamethasone-treated rats resulting in a decrease of NO synthase activity (258)**.** Dexamethasone is a synthetic glucocorticoid, which does not exert mineralocorticoid action, and its inhibiting effects on NO synthase expression were abrogated by a specific GR antagonist. In the same series of experiments, treatment with glucocorticoids attenuated the endothelium- dependent vasodilating effect of acetylcholine but not the endothelium-independent vasodilation induced by penicillamine. Similarly, in studies of healthy subjects, oral cortisol impaired vasodilatation induced by acetylcholine but not sodium nitroprusside, indicating that glucocorticoids affect the endothelial NO system (259)**.**

1.3.5.2 Pro-arrhythmic effects

Glucocorticoids have been increasingly recognised to affect the expression of ion channels in cardiomyocytes; dexamethasone increased the expression of the L-type calcium channels in rat ventricles (260)**.** In another study, dexamethasone down-regulated the $_{Ito}$ density in mouse ventricular myocytes, resulting in prolongation of the action potential duration (261)**.** Conditional cardiomyocyte GR overexpression in a transgenic mouse model was associated with the prolongation of QRS and QTc duration and chronic atrio-ventricular block on the electrocardiogram (ECG) (262)**.** These findings were different from the ventricular arrhythmic events seen after conditional cardiomyocyte MR - overexpression in mice,

indicating that different signaling pathways are activated by stimulation of MRs and GRs in cardiomyocytes. However, it should be noted that as cardiomyocytes lack 11beta-HSD2, MRs are likely to be occupied by endogenous glucocorticoids. Under conditions of oxidative stress and increased production of ROS, cortisol may exert MR agonist rather antagonist effects, mimicking aldosterone effects on ion channels in the myocardium. Indeed, the glucocorticoid corticosterone which binds equally the MR and GR, increased the $_{Ica}$ density of rat cardiomyocytes in vitro similarly to aldosterone when given at low doses (263). These effects were abolished in cardiomyocytes of MR-deficient mice but conserved in GR-deficient mice, indicating that corticosterone (the cortisol equivalent in mice) exerts its effects on calcium current via the MR.

1.3.5.3 Infarct Remodeling

Given their anti-inflammatory effects and lysosomal membrane-stabilising actions, it has been proposed that glucocorticoids might be useful in reducing tissue damage after MI; pre-treatment with methylprednisolone stabilised cardiomyocyte membranes and prevented the leakage of lysosomal enzymes and cell disruption in animals and patients with MI (264) (265)**.** Other studies suggested that treatment with glucocorticoids after MI delays the accumulation of inflammatory cells at the sites of tissue injury; high doses of glucocorticoids after MI inhibited phagocytosis and removal of necrotic cells from the infarct area, resulting in scar thinning and infarct expansion in animal models (266) (267). Moreover, treatment with glucocorticoids has been shown to prevent angiogenesis and to down-regulate neovascularisation reducing the integrity of the scar tissue after MI (268) (269)**.** In keeping with the above studies, in which high therapeutic doses of glucocorticoids were used, urinary levels of cortisol metabolites in patients following acute MI were related to larger infarct volumes and greater infract remodeling over time (270)**.** Moreover, cortisol in doses representing physiological endogenous concentrations increased cardiomyocyte apoptosis

rate and infarct size in an ex-vivo rodent model of MI (271)**.** Interestingly, these effects were mitigated by spironolactone indicating that part of cortisol effects on cardiomyocyte necrosis and infarct size were mediated by MRs.

1.4 Prognostic significance of mineralo- and gluco-corticoids in HF

1.4.1 Aldosterone escape and clinical significance in HF

The concept of aldosterone escape (or breakthrough) describes the failure of suppression of plasma aldosterone levels despite treatment with an ACE inhibitor or ARB (272)**.** The incidence of the phenomenon in HF is variable, with estimates ranging from 10-51% mainly as a result of a lack of consensus on the definition of aldosterone escape (273)**.** Aldosterone breakthrough has been defined as either plasma aldosterone levels above an absolute cut-off or any increase in plasma aldosterone levels from individual baseline levels three to twelve months after initiation of therapy with an ACE inhibitor or ARB. The mechanism of aldosterone escape is likely to be multifactorial and may reflect the importance of trophins other than angiotensin II, such as plasma potassium and ACTH, in the regulation of aldosterone production (274)**.** Non ACE-dependent pathways may also be involved in the pathogenesis of the phenomenon; other proteases as chymase have been shown to generate angiotensin II from angiotensin I in human arteries (275) (276). Moreover, aldosterone escape may promote a vicious cycle in which angiotensin II initially activates aldosterone production, which subsequently up-regulates systematic and tissue ACE activity and furthermore increases aldosterone secretion (220) (277)**.** Furthermore, genetic factors, as the DD genotype of the ACE gene insertion/deletion polymorphism, have also been associated with raised plasma aldosterone levels in patients with HF despite receiving treatment with an ACE inhibitor (278).

It is generally accepted that aldosterone escape is associated with poor clinical outcomes. In patients with HF receiving long term therapy with an ACE inhibitor, aldosterone escape was associated with impaired exercise tolerance and increased ventilatory response during exercise (279)**.** Moreover, in patients with hypertension receiving treatment with an ACE inhibitor or ARB, patients of the escape group showed significant reduction in the LV mass index although the reductions in blood pressure were similar between escape and non-escape groups (280)**.** Aldosterone escape has also been found to be inversely correlated with arterial compliance in patients with HF treated with an ACE inhibitor (281). In addition, markers of insulin resistance were higher in the aldosterone escape compared to the non-escape group in a sub-study of the Aliskiren Observation of heart Failure Treatment (ALOFT) trial (282)**.**

1.4.2 Prognostic significance of plasma aldosterone in patients with HF and interaction with treatment

Apart from the associations with intermediate phenotypes, studies have examined aldosterone levels in relation to mortality and morbidity. Increased levels of plasma aldosterone have been associated with worse prognosis in the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) (55)**.** In this trial, patients with severe HF were randomised to receive treatment with enalapril or placebo in addition to conventional therapy with diuretics, digoxin and aldosterone blockers. Aldosterone levels at baseline were significantly higher in non-survivors compared to survivors at 6 months in the placebo arm. In addition, aldosterone levels were positively correlated with mortality in patients taking placebo but not enalapril. Interestingly, the reduction in medium-term mortality risk with enalapril was prominent in patients with high, but not in patients with low angiotensin II and aldosterone levels at baseline.

The prognostic significance of aldosterone was also examined in the Survival and Ventricular Enlargement (SAVE) study, where patients with an MI and LVSD were randomised to receive captopril or placebo (283). Higher plasma aldosterone levels at baseline were univariately associated with cardiovascular mortality during the follow-up period. Moreover, aldosterone levels were independently associated with the combined end point of cardiovascular mortality, development of HF or recurrent MI in these patients. Plasma neurohormone levels were further measured at three months, one and two years after the index MI in these patients (284). Aldosterone levels were significantly lower in the captopril compared with the placebo group in asymptomatic patients three months post-infarction. In addition, they were independently correlated with the development of severe HF and the combined end point of death or severe HF or recurrent MI during the follow-up period. Interestingly, plasma aldosterone levels remained elevated in patients randomised to placebo, especially in those who had a combined end point event within twenty-four months after the MI. In contrast, there was a decrease in aldosterone levels in patients treated with captopril, especially in those who did not have an event.

In the modern era of HF treatment with an ACE inhibitor and beta-blocker, plasma aldosterone levels were lower in patients with chronic HF and LVSD receiving an ACE inhibitor or a beta-blocker compared with patients taking none of the previous agents in the Valsartan in Heart Failure Trial (Val-HeFT) study (285). Higher aldosterone levels at baseline were univariately associated with all-cause mortality and the combined end point of mortality and morbidity. In a multivariate analysis, a trend of higher mortality and morbidity was present in patients with higher aldosterone levels in this study.

Unfortunately, the Randomised Aldactone Evaluation Study (RALES) (286) and the EPHESUS study (287)**,** which examined the use of aldosterone antagonists in patients with

HF, have not resulted in any publication with regards to aldosterone levels and outcomes so far. In a RALES substudy, plasma aldosterone levels were significantly higher in patients receiving spironolactone at three and six months (288). Conversely, there was a significant decrease in the levels of other neurohumoral markers including BNP in these patients. The reasons for the higher aldosterone levels following aldosterone blockade are not clear. Aldosterone increases via a positive feedback circuit tissue ACE activity, which in turn results in further angiotensin II generation and stimulation of aldosterone secretion (88) (89)**.** Additionally spironolactone might increase directly aldosterone levels by blocking the binding of aldosterone to the MRs.

1.4.3 Prognostic significance of plasma cortisol levels in HF

The data with regards to cortisol secretion in patients with HF are sparse and the prognostic value of glucocorticoids in chronic HF has not been examined until recently. In a study of patients with HF serum cortisol levels were independently linked with all-cause mortality (72). This study also revealed complementary and incremental prognostic value of cortisol and aldosterone when these corticosteroids were examined in combination. In another study, serum levels of cortisol were significantly higher in patients with cardiac events, which were defined as cardiovascular mortality or hospitalisation for HF and were independently associated with worse prognosis (73). This study further examined the impact of oxidative stress, as reflected by plasma oxidised LDL (oxLDL) levels, on the prognostic value of cortisol; patients with high cortisol and oxLDL levels had higher risk of cardiac events compared with patients with high cortisol and low oxLDL levels. Interestingly, there was no significant difference in terms of risk prediction in patients with high cortisol and low oxLDL compared with patients with low cortisol levels.

Overall, the worse outcomes in patients with raised cortisol levels in these studies were attributed to the activation of cardiomyocyte MRs by glucocorticoids, as under circumstances of altered redox state cortisol becomes MR agonist in tissues lacking 11beta-HSD2 (66)**.**

1.4.4 MR blocking in HF – Evidence from clinical trials

In RALES, patients with severe HF and LVSD (LVEF ≤35%) were randomly assigned to receive, spironolactone or placebo, in combination with standard medical therapy (286)**.** The study showed that MR blockade with spironolactone was associated with a 30% reduction in all-cause mortality, irrespective of the HF aetiology. That was due to a lower rate of death from progressive pump failure and sudden cardiac death. Various mechanisms are likely to contribute to the mortality benefit of aldosterone blockade as discussed in section 1.3. Spironolactone opposes the classic epithelial aldosterone effects on sodium retention, improves potassium and magnesium homeostasis and contributes to the reduction of arrhythmias related to potassium and magnesium loss (250) (289)**.** Moreover, aldosterone blockade improves endothelial function and heart rate variability, suppresses vascular inflammation and exerts antifibrotic effects**.** The RALES study group, looking for a mechanistic link to explain the beneficial effects of aldosterone blockade, examined the associations between collagen turnover markers (PINP, PIIINP and PCIP) with all-cause mortality and the interaction between these markers and the effect of spironolactone on outcomes in a subgroup of the RALES cohort (241). Higher PIIINP levels at baseline were associated with increased risk of all-cause death. Moreover, aldosterone blockade significantly decreased PIIINP and PINP levels at six months. Finally, the mortality benefit with spironolactone was more prominent in patients with higher levels of collagen turnover markers at baseline, indicating that the attenuation of myocardial fibrosis contributes to the therapeutic benefit of aldosterone blockade in these patients.

Aldosterone blockade with eplerenone, a selective MR blocker, was further examined in patients with MI complicated with LVSD in EPHESUS trial (287)**.** In this study, the addition of eplerenone was examined on top of standard medical therapy with an ACE inhibitor and beta-blocker, aspirin, statin and coronary reperfusion, three to fourteen days after a complicated MI. Aldosterone blockade was associated with 15% decrease in all-cause mortality. There was also a decrease in sudden cardiac death and all-cause cardiovascular hospitalisation by 21% and 15% respectively. Similar to RALES, aldosterone antagonism with eplerenone reduced collagen turnover markers post-infarct (290)**.** Moreover, much of the benefit of MR blockade in EPHESUS was due to a significant reduction of sudden cardiac death early after randomisation. At 30 days eplerenone reduced all-cause mortality by 31% and sudden cardiac death by 37% (251).

The EMPHASIS (Eplerenone in Mild Patients Hospitalisation and Survival Study in Heart Failure) trial in patients with mild to moderate HF and LSVD has recently filled the knowledge gap about use of aldosterone antagonists in patients with mild to moderate HF with LVSD (291). The EMPHASIS study recruited patients with New York Heart Association (NYHA) II functional class HF and LVEF of no more than 35%. In this study, eplerenone in addition to standard therapy with an ACE inhibitor or ARB and beta-blocker, resulted in 37% reduction in the primary end point of death from cardiovascular causes or hospitalisation for HF and a 35% reduction in the combined end point of all-cause mortality or HF hospitalisation. Additionally, there was also a 42% and 31% reduction in hospitalisation for HF and cardiovascular causes respectively.

Evidence regarding the effects of aldosterone antagonists in chronic HF with preserved EF is substantially lacking at the moment. The Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) trial has been designed to examine the

effects of MR blockade in patients with chronic HF and LVEF ≥ 45% (292). The results of the above trial are currently awaited.

1.5 *CYP11B2* polymorphisms and mineralo- and gluco-corticoid secretion

1.5.1 *CYP11B2* characteristics

Aldosterone synthase is encoded by the *CYP11B2* gene, which lies on chromosome 8q21-22, in close proximity with 11beta-hydroxylase gene (*CYP11B1)* approximately 40 kilobases apart (Figure 1-7). Both genes consist of 9 exons and 8 introns and share 95% and 90% sequence homology within their exonic and intronic regions, respectively (293)**.** *CYP11B1* encodes the enzyme 11beta-hydroxylase, which mainly catalyses the formation of cortisol from 11-deoxycortisol in ZF. *CYP11B2*, encodes aldosterone synthase which catalyses the synthesis of aldosterone from DOC in the ZG. These enzymes are 93% identical, reflecting their shared 11beta-hydroxylase and 18-hydroxylation activity (78). However, differences in the promoter regulatory region of these genes, account for the different pattern of expression and distinct regulatory pathways (294). *CYP11B1* is expressed throughout the adrenal cortex and its transcription is regulated by the ACTH. CYP11B2 is expressed only in the ZG in and its transcription is principally regulated by the angiotensin II.

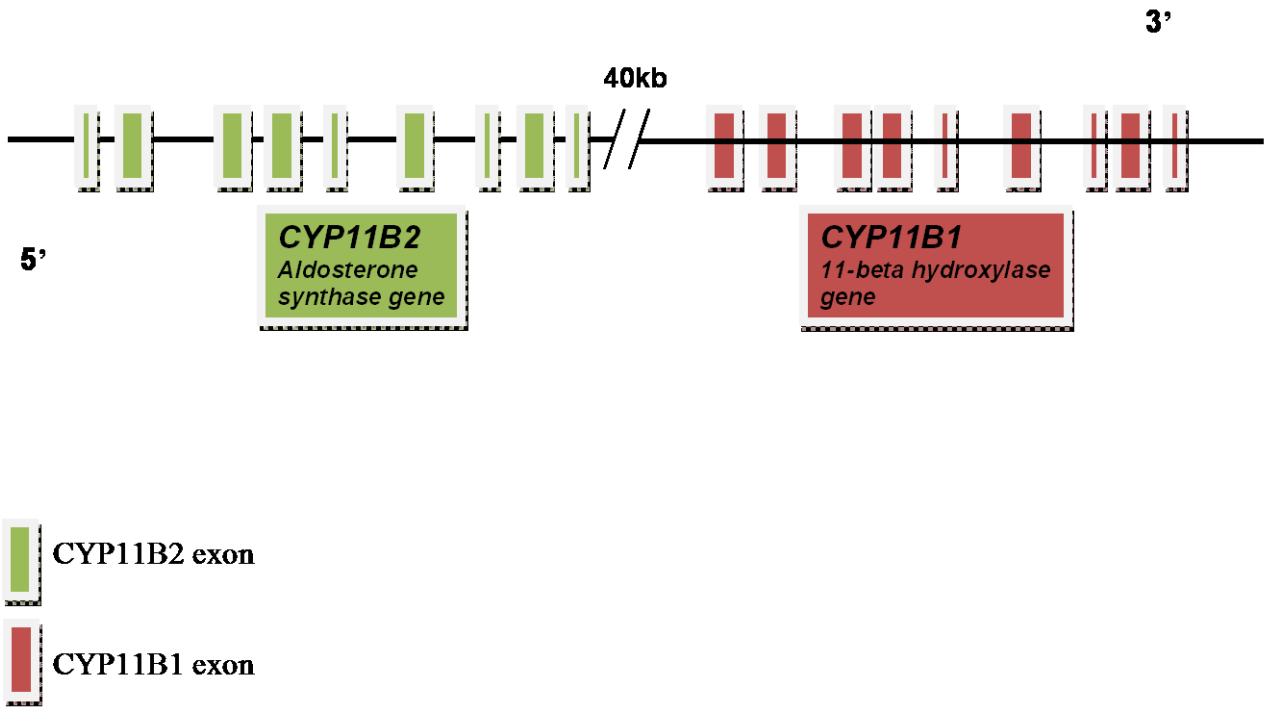


Figure 1-7. Aldosterone synthase gene*(CYP11B2)* and 11beta-hydroxylase gene *(CYP11B1)*

1.5.2 *CYP11B2* polymorphisms associated with aldosterone synthase activity and mineralocorticoid secretion

Several polymorphisms have been described in *CYP11B2* and two of them, one in the transcriptional regulatory region and the other in intron 2, have been extensively studied in relation to mineralocorticoid secretion. The -344C/T polymorphism is located in the 5' promoter region of *CYP11B2* and results in a cytosine/ thymine (C/T) amino acid substitution at position -344 (295). The above polymorphism is located within a putative steroidogenic factor-1(SF-1) binding site, which has been implicated in the expression of adrenocortical steroidogenic enzymes (293). The -344T allele disrupts the transcription binding site and has been shown to bind the SF-1 4 to 5 times less than the C allele (296). However, the T and C alleles exert similar transcription rates in vitro and the SF1 binding site can be deleted

without having an effect on *CYP11B2* transcription, indicating that SF-1 exerts a non-dominant role in the regulation of *CYP11B2* expression (297). Another well studied *CYP11B2* polymorphism is IC, which is an intron conversion caused by an exchange of "wild type''(Wt) intron 2 in *CYP11B2* with the corresponding intron in *CYP11B1* (Con).
The -344T/C polymorphism and the IC have been found to be in tight linkage disequilibrium (LD), defining three different haplotypes (T/Con, C/Wt and T/Wt) (298).

In clinical studies, aldosterone synthase activity has been indirectly examined by measuring aldosterone levels. Aldosterone synthase mediates the final three steps converting DOC to aldosterone in ZG and for that reason a more indicative marker of enzyme activity would be the DOC to aldosterone ratio. However, as DOC takes part in both mineralocorticoid and glucocorticoid synthesis pathways, plasma or urine aldosterone levels and plasma aldosterone to renin ratio have been extensively used to estimate aldosterone synthase activity in vivo instead. In these studies, the *CYP11B2* -344T/C polymorphism has been associated with plasma aldosterone levels, aldosterone to renin ratio and urine excretion rates of aldosterone metabolites in healthy subjects and subjects with hypertension. In normotensive subjects of Caucasian origin 24-hour urine exertion rates of aldosterone metabolites were higher in carriers of the T allele compared with those lacking this allele (299). Correspondingly, in another study with healthy subjects, T allele carriers were found to have higher plasma aldosterone levels than CC homozygotes (300). In a multi-ethnic population of middle aged subjects with normal and high blood pressure, the T allele has been associated with higher plasma aldosterone levels (301). The C allele was more frequent in Caucasian and South Asian patients than individuals of African-American origin; the different allele frequencies between ethnic groups in this study, however, did not affect the associations between -344T allele and plasma aldosterone levels. Moreover, in another study, hypertensive individuals with a raised aldosterone to renin ratio had higher proportion of the T allele compared with

hypertensive subjects with low aldosterone to renin ratio (302). Nevertheless, the associations between *CYP11B2* -344T allele and mineralocorticoid levels have not always been consistent; the -344T/C polymorphism has been reported to have no relationship with aldosterone levels (303) or the C instead of the T allele has been associated with raised aldosterone levels (304). The same trend of discordant results has been reported in association studies with regard to the *CYP11B2* IC. The intron conversion (Con) has been associated with higher plasma and urine aldosterone levels in some (298) (305) (306) but not in all studies (307).

From the other *CYP11B2* polymorphisms studied, a variant in exon 3, which results in a lysine/ arginine (L/A) amino acid substitution at residue 173 (K173R), has been shown to be in tight LD with -344T/C polymorphism; a haplotype including -344T and K173 was associated with higher *CYP11B2* expression in adrenal tissue compared to other haplotypes, indicating that the K173R polymorphism may have a causative or a synergistic role with the -344T/C polymorphism with regards to aldosterone synthase efficiency (308). However, although the K173R polymorphism has been associated with low renin hypertension in another population, no obvious effect on enzymatic activity, expressed as the ability to convert DOC to aldosterone, has been demonstrated in vitro (309).

Despite the numerous studies with regards to *CYP11B2* polymorphisms in relation to aldosterone secretion in healthy subjects and hypertensive subjects, little data exist on the interaction between *CYP11B2* polymorphisms and aldosterone secretion in conditions related to activation of the RAAS; specifically, whether polymorphisms of the *CYP11B2* have an impact on aldosterone synthesis in patients with HF remains unclear.

1.5.3 *CYP11B2* and *CYP11B1* polymorphisms associated with 11beta-hydroxylase activity and glucocorticoid secretion

11beta-hydroxylase activity varies within normal subjects and part of this variability is genetically determined (310). Basal and ACTH-stimulated plasma levels of 11-deoxycortisol and DOC and DOC to corticosterone ratio were found to be significantly heritable in this study. 11beta-hydroxylase activity is usually defined by the ratio of plasma 11-deoxycortisol to cortisol or DOC to corticosterone or by the ratio of the urinary excretion rates of their metabolites respectively. An increased 11-deoxycortisol to cortisol ratio implies that more substrate is needed for the enzyme to synthesise the biologically required amounts of cortisol.

The *CYP11B2* promoter polymorphisms have been previously found to be associated with altered 11beta-hydroxylase efficiency in healthy subjects. In a study of male healthy subjects corticosteroid levels were not different at baseline (after a dexamethasone suppression test) between the -344T/C genotypes (295). Cortisol response to ACTH was also unaffected by the -344T/C genotype; however, 11-deoxycortisol levels were significantly higher in TT homozygotes compared to heterozygotes and CC homozygotes after ACTH stimulation, indicating a relative impairment of 11beta-hydroxylase efficiency in these subjects. A similar pattern was identified between the IC genotype groups in these patients; there was no difference in ACTH-stimulated cortisol levels between the genotypes, but 11-deoxycortisol levels increased significantly in response to ACTH in subjects with Con genotype compared to subjects with Wt genotype. Similar differences in corticosteroid levels according to the above *CYP11B2* genotypes were seen in another study that included both male and female healthy subjects (299). Moreover, the above associations were further replicated in patients with essential hypertension. In a subgroup of unrelated subjects of the British Genetics of Hypertension (BRIGHT) study, cortisol and 11-deoxycortisol urine metabolite levels were

not different between *CYP11B2* genotypes (311). However, the ratio of 11-deoxycortisol to total cortisol urine metabolites was significantly higher in TT than CC homozygotes. Similar differences were found among TC compared to CC patients, however the differences among TT and TC patients were not statistically significant.

The correlations between *CYP11B2* polymorphisms and biomarkers of 11beta-hydroxylase efficiency found in these studies were somewhat unexpected at a first sight. Aldosterone synthase does not metabolise 11-deoxycortisol, which is converted to cortisol by the enzyme 11beta-hydroxylase. The *CYP11B1* gene encodes 11beta-hydroxylase and as previously mentioned lies in close proximity to *CYP11B2* gene on chromosome 8 in humans. It was speculated that the quantitative trait locus for the corticosteroid intermediate phenotypes would probably lie within *CYP11B1* gene and that LD across the *CYP11B2*/*CYP11B1* locus could account for these observations (298). In view of this hypothesis, two groups were able to demonstrate the presence of LD between *CYP11B1* and *CYP11B2* promoter regions (312) (313). Both studies found a limited number of frequently occurring haplotypes and further demonstrated that the higher ratio of urinary 11-deoxycortisol to cortisol metabolite excretion rates was correlated with haplotypes carrying the *CYP11B2* -344 T allele.

Further evidence regarding the presence of LD across *CYP11B1*/*CYP11B2* locus was provided by another study, which identified two novel polymorphisms (1889 G/T and 1859 A/G) in the 5' promoter region of the *CYP11B1* (314); haplotype analysis demonstrated tight LD across the entire CYP11B locus revealing four common haplotypes. The -1889T and 1859G alleles were associated with reduced *CYP11B1* transcription in vitro in response to stimulation with agonists. Moreover, in subjects with hypertension a higher urine 11-deoxycortisol to cortisol ratio was evident in homozygotes for the -1889T allele than heterozygotes or homozygotes for -1889G allele. A similar trend was found for the -1859G/T

polymorphism, with the GG patients showing impaired 11beta-hydroxylase efficiency compared with the GT or TT patients. Overall, that study identified novel *CYP11B1* variants and provided strong evidence for LD between polymorphisms in the promoter region of *CYP11B1*/*CYP11B2*. These polymorphisms were also correlated with altered 11beta-hydroxylase activity and might represent the causative loci for the associations seen between the -344T/C polymorphism and markers of 11beta-hydroxylation.

1.6 Prognostic significance of *CYP11B2* -344T/C polymorphism in patients with HF or patients with MI

The association between the *CYP11B2* promoter -344 T/C polymorphism and prognosis has been examined in patients with HF in the Genetic Risk Assessment of Heart Failure in African-Americans (GRAHF) (315), which was a genetic sub-study of the African-American Heart Failure Trial (A-HeFT) (316). In the A-HeFT study, African-Americans with HFrSF were randomised to receive either a fixed combination of isosorbide dinitrate and hydralazine or placebo, in addition to standard therapy with a beta-blocker, ACE inhibitor/ARB or aldosterone blocker. In the GRAHF study, apart from the association between *CYP11B2* -344T/C polymorphism and outcomes, a pharmacogenetic interaction between the above polymorphism and the treatment with regards to prognosis was additionally explored in 354 of the A-HeFT patients. Subjects were followed-up to an end point of death or HF hospitalisation. A composite score, calculated from the combination of all-cause mortality, HF hospitalisation and change in quality of life at six months, was employed as the primary end point.

The *CYP11B2* -344C allele was associated with significantly poorer event-free survival (death or HF hospitalisation) during the course of follow-up in GRAHF (Figure 1-8), with TT

homozygotes having better and CC homozygotes having the worse outcomes. Similarly, patients with the C allele had greater mortality than TT patients. Interestingly, in a pharmacogenetic sub-analysis, homozygotes for the T allele, responded significantly better to isosorbide dinitrate/hydralazine combination therapy with improvement in the composite score at six months than the C allele carriers (TC+CC) and that was mainly driven by improvement in the quality of life score. On the other hand, the -344C allele was linked to LV remodeling in placebo but not in the group treated with the isosorbide dinitrate/ hydralazine combination therapy; In the placebo group, C allele homozygotes had a greater LV end-diastolic diameter (LVEDD) compared with the TT homozygotes at six months. Similarly, a lower LVEF was present at 6 months in CC homozygotes compared with the TT homozygotes in the placebo group but not in patients treated with combination therapy. Overall, the effects of the C allele on LV remodeling and LVEF were diminished by the combination therapy, however, that was not translated in better survival in CC/CT compared with TT patients.

Aldosterone levels were not measured in GRAHF study and a gene additive model based on previous studies in patients with hypertension (304) was a priori implemented to explain the associations between -344T/C polymorphism and outcomes in GRAHF. According to this model, aldosterone levels increase in a stepwise fashion as the number of the C allele increases, and patients with the CC genotype have higher aldosterone levels than patients with the TC and TT genotypes respectively. However, as mentioned in section 1.3.2, the C allele additive model has not always been replicated and several studies associated the T rather the C allele with higher levels of aldosterone (298) (300). Interestingly, the -344T allele has consistently been associated with higher 11-deoxycortisol to cortisol ratio compared with the -344C allele (section 1.3.2). Unfortunately, mineralo- and gluco-corticoid levels were not measured and aldosterone synthase or 11beta-hydroxylase activity was not

assessed in GRAHF or other HF study. Whether *CYP11B2* polymorphisms are associated with mineralo- or gluco-corticoid levels and additionally have an impact on outcomes in patients with HF is unknown.

The association between -344 T/C polymorphism and survival has also been investigated in patients of predominantly Caucasian origin following an MI (317). At baseline TT homozygotes had a higher incidence of antecedent hypertension than TC + CC patients and that was more pronounced for males. Furthermore, patients with the TT genotype had higher levels of BNP, 24-96 hours after the onset of MI. Interestingly patients with the TT genotype had better survival compared to patients with CT and CC genotypes. Similar to the GRAHF study plasma mineralo- and gluco-corticoid levels were not measured in these patients and it was assumed that the association between -344T/C polymorphism and survival represents a pharmacogenetic response to background therapy. Nevertheless, in the absence of plasma or urine corticosteroid measurements, no definitive mechanistic link between *CYP11B2* -344C allele and worse prognosis was identified.

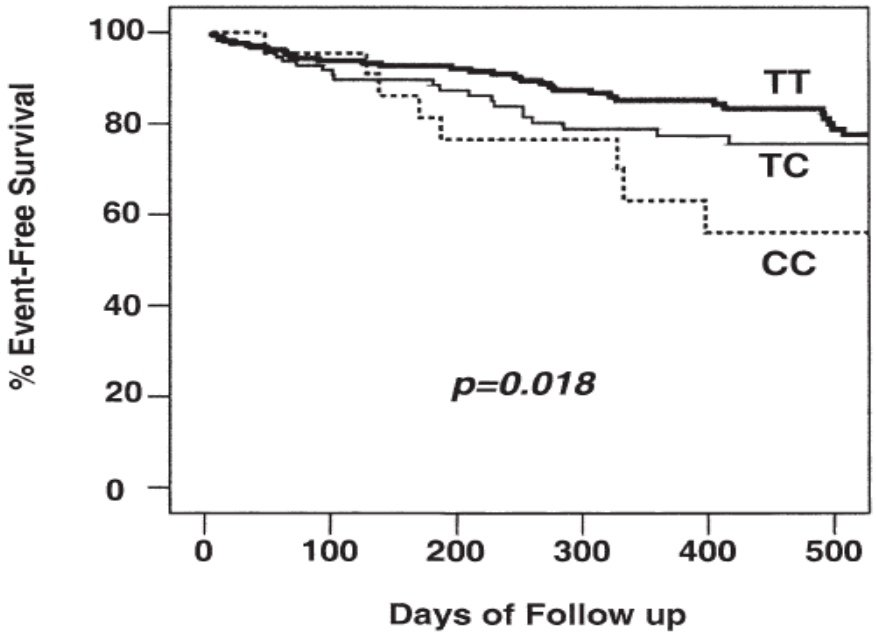


Figure 1-8. Event free survival by *CYP11B2* -344C/T genotypes in patients with severe HF. Adapted from McNamara et al. (315)

1.7 Hypotheses and Aims

The hypotheses tested in the experimental section of this thesis were that:

- RAAS activity and glucocorticoid concentrations are associated with markers of HF severity in patients with acute decompensated and chronic HF.
- The dissociation between RAAS and natriuretic peptide levels seen early after initiation of diuretic therapy in patients with decompensated HF is present in the medium- to long-term.
- Increased RAAS activity and glucocorticoid levels measured during hospital admission are associated with worse prognosis in patients with HF.

- Variants in the *CYP11B2* locus are associated with 11beta-hydroxylase efficiency, gluco- and mineralo-corticoid secretion and survival in patients with decompensated HF.

The aims to test these hypotheses were:

- Comparison studies examining patient characteristics according to renin and aldosterone levels, 11-deoxycortisol and cortisol levels, and aldosterone to renin and 11-deoxycortisol to cortisol ratio in patients with HF not taking a RAAS inhibitor during hospital admission and at the follow-up visit.
- Studies comparing RAAS activity and other laboratory and clinical variables between hospital admission and follow-up in patients not taking a RAAS inhibitor at both time points.
- Survival studies examining the associations between plasma levels of RAAS mediators and glucocorticoid during hospital admission and all-cause mortality in patients with HF.
- Comparison studies examining mineralocorticoid and glucocorticoid levels and other patient characteristics during hospital admission, according to *CYP11B2* -344T/C and IC polymorphisms.
- Survival studies examining associations between *CYP11B2* -344T/C and IC polymorphisms and all-cause mortality in patients with HF.

2. Chapter Two - Methods

2.1 Introduction

In this chapter, I present the methods employed in the studies of this thesis. The identification of the participants, the study design and collection of the data as well as the laboratory analyses performed in these studies will be described in detail.

2.2 Healthy Volunteers studies

2.2.1 Recruitment of healthy subjects

Eight healthy subjects were included in these studies. These were identified

a) From a list of healthy volunteers who had already participated in previous studies in the British Heart Foundation (BHF) Glasgow Cardiovascular Research Centre and expressed an interest to be contacted for participation in future studies.
b) Through advertisement within the University of Glasgow and the Western Infirmary in Glasgow (WIG).

All subjects who expressed an interest in participating in the study were invited to attend the clinical research unit at the BHF Glasgow Cardiovascular Research Centre and an information leaflet was provided. Subjects were given time to decide whether they wished to take part in the study and those who remained agreeable were asked to sign a consent form in the presence of the research doctor at the BHF Glasgow Cardiovascular Research Centre.

2.2.1.1 Screening Visit

In the screening visit, a routine clinical examination was performed and a 12-lead ECG and a blood sample for routine kidney function tests were undertaken. Blood pressure measurements were performed after 5 minutes of rest in a sitting position. A standard sphygmomanometer was used and the correct cuff size was selected according to the arm circumference of the participant. Volunteers were asked to complete a questionnaire to ensure that they did not suffer from any significant chronic disease.

Exclusion criteria for participation were as follows:

1) Age less than 18 years or greater than 50 years

2) Blood pressure >160/90 mmHg

3) Cardiovascular or chronic disease

4) Chronic medication

5) History of allergy or asthma

6) Drug abuse

7) Pregnancy

8) Abnormal kidney function, which was defined as eGFR < 60ml/min/1.73m^2 and creatinine >98 µmol/L for women and >120 µmol/L for men.

9) Inability to comply with the study instructions

2.2.1.2 Study protocol

The study included four visits and the time interval between the visits was around seven days.

Visit 1 − Ambulatory blood sampling

Healthy subjects attended the BHF Glasgow Cardiovascular Research Centre at 7.30 am. An intravenous (IV) cannula was inserted in a forearm vein on arrival and subjects rested supine

for 30 minutes before the first blood samples were taken at 8.00am. Ambulatory blood samples were taken at 10.00am, 12.00pm, 4.00pm, 8.00pm and 10.00pm for PRC and plasma gluco- and mineralo-corticoid measurements.

Visit 2 − ACTH stimulation test

Subjects were asked to take 1 mg of oral dexamethasone (Pharmacy Unit, NHS Greater Glasgow and Clyde) twice daily on day 2 and 3 and to collect a 24-hour urine sample on day 3 for urinary electrolyte measurements. On day 4, an ACTH stimulation test was performed. Subjects had an IV cannula inserted in a forearm vein at 7.00am, and rest supine for 60 minutes. 1 μg of synthetic ACTH (Pharmacy Production Unit, NHS Greater Glasgow and Clyde) was slowly injected and blood samples were taken at 0, 10, 30 and 60 minutes for PRC and plasma mineralo- and gluco-corticoid measurements.

Visit 3 − Low salt diet / Angiotensin II infusion

Healthy subjects were given a diet sheet and advice to achieve controlled sodium diet (80 mmol/day) for three days (day 1, 2, 3) before their visit to the BHF Glasgow Cardiovascular Research Centre for visit 3 (day 4). Subjects were asked to take a 40 mg once-only dose of oral furosemide (Pharmacy Unit, NHS Greater Glasgow and Clyde) and to collect a 24-hour urine sample for urinary electrolyte measurements on day 3. On day 4, an angiotensin II infusion study was performed. After 60 minutes of supine rest starting at 7.00am, angiotensin II (Merck, Läufelfingen, Switzerland) was infused via an indwelling IV cannula in the forearm at 1, 3 and 5 ng/kg/min for 20 minutes at each dose. Blood samples for PRC and plasma mineralo- and gluco-corticoid measurements were removed through an indwelling IV cannula in the opposite forearm at 0, 20, 40 and 60 minutes. The systolic blood pressure (SBP) and diastolic blood pressure (DBP), mean arterial pressure (MAP) and pulse rate were

recorded every 5 minutes. The infusion was discontinued if there was more than 20 mmHg increase in the MAP.

Visit 4 − High salt diet / Angiotensin II infusion

Healthy subjects were given a diet sheet and advice to follow controlled sodium diet (120 mmol/day) and take slow sodium tablets (100 mmol per day) for three days (day 1, 2, 3) before their visit to the BHF Glasgow Cardiovascular Research Centre for visit 4 (day 4). Subjects were asked to collect a 24-hour urine sample for urinary electrolyte measurements on day 3. On day 4, an angiotensin II infusion study was performed. After 60 minutes of supine rest starting at 8.00am, angiotensin II was infused via an indwelling IV cannula in the forearm at 1, 3 and 5 ng/kg/min for 20 minutes at each dose. Blood samples for PRC and plasma mineralo- and gluco-corticoid measurements were removed through an indwelling IV cannula in the opposite forearm at 0, 20, 40 and 60 minutes. The SBP and DBP, MAP and pulse rate were recorded every 5 minutes. The infusion was discontinued if there was more than 20 mmHg increase in the MAP.

The study protocol was approved by the West Glasgow Ethics Committee and all healthy volunteers provided a written informed consent.

2.2.2 Laboratory measurements

Plasma mineralo- and gluco-corticoids were measured by the MRC Blood Pressure Group (Ms M Ingram and Prof R Fraser) at the BHF Glasgow Cardiovascular Research Centre using a Liquid Chromatography Mass Spectrometry (LCMS) method. Three milliliters (ml) of plasma, containing an internal standard (16β-methylprednisone: 60 ng) were added to a Chem Elut SPE cartridge (Varian Inc.) and allowed to stand for at least 5 minutes. The steroids were eluted with dichloromethane (high-performance liquid chromatography [HPLC] grade:

2 x 5 ml) and the eluate was evaporated until dry under nitrogen. The residue dissolved in 10% acetonitrile (HPLC grade: 60 µl) of which 20µl was applied to the column (Polaris^{TM}, 5 µ C18-A, 150 x 20 mm), which was developed by gradient elution (acetonitrile:water containing 2 mmol ammonium acetate). Finally, the column was coupled to a mass spectrometer (Varian 1200L with a triple quadropole detector).

PRC was analysed by the Diasorin chemiluminescent immunoassay using the Liaison platform (Diasorin S.p.A, Saluggia, Italy) at the Glasgow Royal Infirmary (GRI) (Prof M Wallace). Urinary sodium and potassium excretion rates were measured on 24-hour urine collections by the Department of Biochemistry at the WIG using an ion-selective electrode method.

2.3 HF study – Hospital admission

2.3.1 Study design and participants

Almost all patients admitted to the WIG and GRI with decompensated HF between July 2007 and January 2009 and the Royal Alexandra Hospital (RAH) in Paisley between April 2008 and January 2009, were screened (Dr Y Tsorlalis at WIG, Dr C Jackson at GRI and research nurse team at RAH) prospectively for inclusion in this study. The majority of patients were screened in the acute medical assessment and coronary care unit or the cardiology ward of each hospital within 24 - 72 hours of hospital admission. Screening involved looking daily at the case notes of all the new admissions.

The inclusion criteria for participation in the study were as follows:

1. Admission with symptoms and signs of HF
2. Radiological evidence of HF
3. Response to IV diuretics

Patients were eligible for screening and potential inclusion in the study, if they were admitted to hospital with symptoms and signs of HF and had radiological evidence of HF or responded to IVdiuretics.

The principal exclusion criteria are listed below:

- Acute coronary syndrome complicated by pulmonary oedema
- Serious concurrent systemic disease
- Cognitive impairment

- Geographical or social reasons making study visit not feasible

All the eligible patients were approached for enrollment in the study, which involved two stages. At the first stage, patients expressing an interest to participate in the study were provided with an information sheet and they were asked to give written permission for blood sampling to measure BNP, PRC and plasma corticosteroids and to extract DNA for identification of *CYP11B2* polymorphisms. In addition, permission was given for access to the patients' medical records in order information to be recorded by the medical and nursing stuff recruiting for the study. Finally, patients consented at this stage to be followed-up through the Information Services Division (ISD) of the National Scottish Health Service with regards to death recording.

The BNP result was available within 24 hours after the blood sampling and patients were informed about that the following day. Patients with BNP <100 pg/ml were not asked to participate in the next stage of study and the measurements of PRC and corticosteroids and the genotyping for *CYP11B2* polymorphisms were not performed. Patients with BNP ≥100 pg/ml were invited to visit the BHF Glasgow Cardiovascular Research Centre in approximately 4 - 6 weeks following their discharge from hospital. Following the consent form for the second stage, an appointment with date and time was arranged for every surviving patient prior to discharge. The study was approved by the Greater Glasgow & Clyde Ethics Committee.

Demographic data, medical history, physiological measurements, 12-lead ECG findings and transthoracic echocardiogram parameters, medication and laboratory measurements were recorded in all patients enrolled in the study during hospital admission. Demographic data included age and gender. The medical history involved history of previous HF, MI, angina,

diabetes mellitus, hypertension, atrial fibrillation (AF) and cerebrovascular accident (CVA). The pulse rate, SBP and DBP measured on admission were also recorded as part of the physiological measurements. Every patient had their weight and height measured and their BMI was calculated. A 12-lead ECG was performed in all patients at the time of admission and the heart rhythm was recorded.

Most of the patients had a transthoracic echocardiogram performed during the index admission or early after discharge. The LVEDD as well as the presence or absence of dilated left ventricle was recorded. In addition, the presence or absence of LV hypertrophy (LVH) was recorded according to the echocardiogram reports in each hospital (patients with interventricular septal thickness or LV posterior wall thickness >12 mm at the end of diastole in 2-dimensional or M-mode measurements were classified as having LVH). Calculations of the LVEF were not regularly carried out during hospital admission. The assessment of LV systolic function was based on qualitative assessment instead and whether there was LVSD or not was documented.

The cardiovascular and oral glucocorticoid therapy prior to admission and the HF medication during the first 24 hours of admission were documented for every patient enrolled in the study.

2.3.2 Blood sampling and laboratory measurements

Blood sampling for neurohumoral and corticosteroid measurements was undertaken within 24 - 72 hours following hospital admission. Most of the patients admitted to hospital between Monday morning and Friday midday had blood samples collected within 24 hours and patients admitted to hospital between Friday midday and Monday morning had blood samples collected within 24-72 hours following the hospital admission. All blood samples were collected in the morning, between 8am and 11am, with the majority of patients resting in the semi-recumbent position for at least 20 minutes. Blood samples for BNP and PRC were collected in tubes containing potassium ethylenediaminetetraacetic acid (EDTA). Blood samples for aldosterone, cortisol and 11-deoxycortisol were collected in lithium-heparin tubes. The EDTA tubes for BNP were sent to Gartneval General Hospital in Glasgow and analysed (Dr R Spooner) using the Architect Assay (Abbott Laboratories, Abbott Park, IL, USA). The EDTA and lithium-heparin tubes for PRC and corticosteroid measurements were transferred to the BHF Glasgow Cardiovascular Research Centre within 4 - 6 hours of blood sampling and centrifuged at 4 ^{o}C for 20 minutes. The supernatant was immediately frozen and stored at -80 ^{o}C in the BHF Glasgow Cardiovascular Research Centre until later blinded batched analysis. All samples were subjected to one freeze-thaw cycle only. Frozen PRC aliquots were sent to the GRI and measured (Prof M Wallace) by the Diasorin chemiluminescent immunoassay using the Liaison automated platform (Diasorin S.p.A, Saluggia, Italy). The assay working range is 5 - 500 mIU/L and samples containing PRC above 500 mIU/L were diluted in a diluent supplied by Diasorin (code - Endo 31933) (318). PRC values below 5 mIU/L were recorded as 5 mIU/L for the purposes of analyses.

Three milliliters of stored plasma were analysed for aldosterone, cortisol and 11-deoxycortisol levels by LCMS in the BHF Glasgow Cardiovascular Research Centre (Ms M

Ingram and Prof R Fraser). The methodology used for the analysis of corticosteroids by LCMS was described in section 2.2.2.

Stored plasma aliquots for PRC and corticosteroids were available and adequate for analyses for most of the patients recruited in the study (Table 2-1 below). These aliquots were selected and analysed for PRC and corticosteroids only by availability and adequacy criteria and this selection was essentially random.

Table 2-1. Number and percentage (%) of patients with each variable measured in the overall hospitalised cohort (n=722).

Variable	Number of patients with each variable measured	Percentage (%) of patients with each variable measured
PRC	689	95.4
Aldosterone	551	76.3
Cortisol	613	84.9
11-deoxycortisol	600	83.1

Blood samples for routine biochemical and haematological tests were taken during hospital admission and analysed as part of the routine practice in the Biochemistry and Haematology laboratories. All patients had urea and electrolytes (U&Es), and full blood count (FBC) analysed and the majority of patients had troponin measured at the time of hospital admission. The estimated glomerular filtration rate (eGFR) was calculated using the

Modification of Diet in Renal Disease (MDRD) equation[1]. Troponin I was measured at the WIG and GRI by the Architect assay (Abbot Laboratories, Abbott Park, IL, USA) and troponin T was measured at the RAH by the Roche assay (Roche Diagnostics, Basel, Switzerland). Elevated troponin I and T results were reported as ≥ 0.04 μg/L and ≥ 0.05 μg/L respectively.

CRP, thyroid stimulating hormone (TSH) and lipid profile measurements were additionally recorded if tests were performed during admission.

2.3.3 Genotyping

2.3.3.1 Extraction and quantification of genomic DNA

10 ml of EDTA-preserved whole blood was added to 40 ml of cell lysis mix in a universal tube, which was left on ice for 10 minutes before centrifugation at 1660 x *g* for 10mins at 4°C. The resulting pellet was re-suspended in 3 ml of nucleic lysis mix. 200 μl 10% sodium dodecyl sulphate (SDS) and 100 μl proteinase K (10 mg/ml) were added and the tubes were left for incubation overnight at 37°C. Following incubation, 1 ml of 6 M $NaCl_2$ was added with vigorous shaking and after addition of 5 ml of phenol:chloroform:isoamyl alcohol (25:24:1) the tubes were centrifuged at 1660 x *g* for 20 minutes at 4°C. The upper phase of the supernatant was removed and two volumes of ethanol were added. DNA was spooled out with a glass rod, washed in 70% (v/v) ethanol and allowed to air dry; it was then suspended in 100μl Tris-HCL-EDTA buffer (TE) before storage at 4°C. The extraction of DNAwas carried out by Dr G Inglis

---

[1] eGFR (ml/min/1.73m$^{2)}$ = 32788 x (serum creatinine in mmol/L)$^{-1.154}$ x (Age) $^{-0.203}$ x (0.742 if female) x (1.212 if African American/Caribbean)

1 µl of DNA solution was mixed with 1 ml of distilled water in a quartz cuvette, which was inserted in a dual beam spectrophotometer. DNA concentration was determined according to the following equation:

$[DNA] = 50 \times Absorbance_{260nm}$

Absorbance refers to the wavelength of light that is absorbed by DNA at 260nm and was used to determine the DNA concentration in each sample. The quantification of genomic DNA was performed by Dr Y Tsorlalis and Mrs C Holloway

2.3.3.2 *CYP11B2* IC Genotyping − Polymerase chain reaction (PCR) − amplification and automated sequencing

PCR amplification protocol

The reaction mix was prepared in sterile eppendorf tubes on ice.

10 x Thermo-Start DNA polymerase buffer	250µl
$MgCl_2$ (25 mM)	200µl
dNTPs	500µl
Sense oligonucleotide primer (ICTAQMAN F)	100µl
Sequence: 5' GATGGCATGAAGCACAAAGCT 3'	
Antisense oligonucleotide primer (ICTAQMAN R)	100µl
Sequence: 5' CCTTGGGCGACAGCACA 3'	
Enzyme (Taq ABGene)	12.5 µl
Nuclease free (NF) water (Ambion, UK)	337.5

The reagents were kept on ice and 15 µl of this mastermix added to 10 µl of DNA (5ng/µl), which had been pre-plated on to 96-well plate. A 96-well DNA Engine Dyad thermocycler (MJ Research, USA) was used for the amplification reaction according to the following thermocycling protocol:

1. 95°C for 15 minutes
2. 95°C for 15 seconds
3. 62°C for 30 seconds
4. 72°C for 2 minutes
5. Repeat step 2–4 x 35 more cycles
6. 72°C for 7 minutes
7. Incubate at 4°C for ∞

Identification of PCR products - Agarose gel electrophoresis

A 1 % (w/v) tris/borate/EDTA (TBE) - agarose gel was prepared by adding 1 g of agarose (Eurogentec, Belgium) in 100 ml of TBE buffer (Sigma-Aldrich, UK) and melting it in a 950W microwave oven for 60 seconds. After cooling for a few minutes, the mix was transferred in a fume hood where 1 µl of ethidium bromide (EtBr) (Sigma-Aldrich, UK) was further added. Agarose, was then effused into a gel mould with Teflon combs and allowed for 15 minutes for the gel to be formed. After removing the combs, the gel was placed in a standard electrophoresis tank containing TBE buffer (Bio-rad, UK). 10 µl of each PCR reaction product with 5 µl of loading dye were loaded in separate gel wells. Subsequently the gel was resolved at 80 volts for 50 minutes. DNA bands on the gel were visualised by an ultraviolet trans-illuminator (Fluor-S MultiImager, Biorad, UK) and images obtained with Multi-Analyst software (Biorad, UK)

PCR product clean-up

The AMPure (Agencourt, USA) purification method was used for the PCR product clean-up. The PCR plates were carefully unsealed and 36 µl of AMPure was added to each well. The plate was then sealed and centrifuged at 210 x *g* for 30 seconds. After incubation for 3 minutes at room temperature, it was placed on a solid phase reversible immobilisation (SPRI) magnet for 10 minutes. Afterwards the plate while being on magnet was inverted on a paper to remove the supernatant. Subsequently, 200 µl of 70% ethanol was added to each well and after 30 seconds of incubation the plate inverted on a paper tissue again to discard the solution before being centrifuged at 76 x *g* for 30 seconds. The magnet was removed from the plate, which was dried in room temperature for approximately 20 minutes. 40 µl of nuclease free water was added in each well and the plate was replaced on the magnet for 5 minutes. 10 µl of the clear product was transferred to a new 96 well plate for the sequencing reaction.

Sequencing reaction set-up

The plate was used for the sequencing reactions containing the following in each well:

PCR product	(10.0 µl)
Sequencing buffer	(3.5 µl)
Ready reaction mix	(0.5 µl)
Sequencing primer - INTCONR(B1B2)	(1.0 µl)
Sequence: 5' GTGTTCGAGCTGCAGCCTTTT 3'	
Nuclease free water	(5.0 µl)

The resultant reaction mix was subjected to cycle sequencing using a 96-well Dyad Disciple sample block powered by the PTC-0021 thermal cycler with heated lid (MJ Research Waltham, MA, USA.) according to the following thermocycling conditions:

1. 96°C for 45 seconds
2. 60°C for 4 minutes
3. Repeat step 1–2 x 25 more cycles
4. Incubate at 4°C for ∞

Sequencing reaction clean-up

A second clean-up was performed using CleanSEQ (Agencourt, USA). Each sequencing reaction was mixed with 10 µl of CleanSEQ and 62 µl of 85% ethanol and an adhesive paper lid used to seal the plate. After incubation at room temperature for 15 minutes, the plates were centrifuged for 30 seconds at 210 x *g* and placed on the SPRI magnet for 10 minutes. The seal was removed and the PCR plate was inverted onto a paper tissue to discard the solution. Subsequently 150 µl of 85% ethanol was added to each well and after 30 seconds of incubation the plate was inverted again on paper tissue to remove the supernatant and centrifuged at 210 x *g* for 30 seconds. Afterwards, the plate was left at room temperature to dry for approximately 20 minutes. 40 µl of nuclease free water were added to each sequencing reaction and the plate was placed on a magnet again after being centrifuged at 210 x *g* for 30 seconds. 20µl of sequence product were transferred to each well of a barcoded plate for the Big Dye Sequencer.

Automated Cycle Sequencing

Sequencing was performed using the Applied Biosystems BigDye Terminator v3.1. cycle sequencing kit (PE Applied Biosystems, CA,USA).The sequencing reaction results were visualised using SeqScape Version 2.1.1.

CYP11B2 IC genotyping was carried out by Dr Y Tsorlalis and Mrs C Holloway

2.3.3.3 *CYP11B2* -344 T/C Genotyping: Taqman method

1 µl of each DNA sample (5ng/µl) was plated on to a Micro Amp Optical 96-well reaction plate (Applied Biosystems, U.K.). 1 µl (concentration 5ng/µl) of DNA, genotyped for the -344 T/C polymorphism (positive controls) and 1 µl of nuclease free water (negative controls) were added to the designated locations in the plate. The plate was sealed and centrifuged at 210 x *g* for 1 minute.

The reaction mix for the *CYP11B2* -344 T/C genotyping with the Taqman method was prepared using probes and primers that were made to order by Life Technologies, CA, USA (Assay ID: C_8896484_10)

TaqMan Genotyping Master Mix (Applied Biosystems CA, USA)	560µl
Primers & probes	14µl
NF water (Ambion, UK)	434µl

9 µl of master mix were added to the plate, which had been pre-plated with 1 µl of DNA. Therma seal RT film for Real-time PCR (Excel Scientific, Wrightwood CA, USA) used for

the plate sealing, which was subsequently placed on the High-Speed Microplate Shaker (Illumina, USA) and pulsed at 480 x *g* for 10 seconds. Afterwards, the plate was centrifuged at 210 x *g* for 1 minute.

The amplification reaction was performed on a 96-well AlphaTM sample block powered by the PTC-225 Engine Tetrad(R) Cycler with heated lid (MJ Research, Waltham, MA, USA) according to the following cycling parameters:

1. 95°C for 15 minutes
2. 95°C for 15 seconds
3. 60°C for 1 minute
4. Go to step 2, 49 times
5. 10°C forever

Following the PCR, the genotypes were analysed by the ABI Prism 7900 HT. Separate clusters corresponding to different genotypes were identified by the SDS 2.3 software (Applied Biosystems). Samples which either were not assigned clearly in a separate cluster or characterised by a quality value <95% were not analysed. Finally, in order to exclude any incorrect analyses by the software, the results were further read by the operator (Dr Y Tsorlalis and Mrs C Holloway)

2.4 HF study – follow-up visit

2.4.1 Study design

Surviving patients returned for follow-up at the BHF Glasgow Cardiovascular Research Centre approximately 4 - 6 weeks following their discharge from hospital, in the afternoon

between 12 - 3pm. The follow-up visits commenced in September 2007 and were completed in March 2009.

Similar to the variables recorded during hospital admission, demographic data, physiological measurements and 12-lead ECG findings were recorded for all patients during follow-up. All the cardiovascular medications and the treatment with an oral glucocorticoid were documented during follow-up. All patients had a transthoracic echocardiogram (Acuson Sequoia C512) in the BHF Glasgow Cardiovascular Research Centre by a single operator (Dr C Jackson). The LVEF could be calculated for the majority of patients using the Simpson's method by a single cardiac echocardiography physiologist (Mr T Cunningham).

2.4.2 Blood sampling and laboratory measurements

Blood sampling for neurohumoral and corticosteroid measurements was undertaken at the BHF Glasgow Cardiovascular Research Centre between 1pm and 4pm, with the patients resting in the sitting position for at least 20 minutes. Similar to the hospital admission, blood samples were collected for PRC, BNP, aldosterone and 11-deoxycortisol and cortisol. The same methodology (section 2.3.2) was employed with regards to the centrifugation, extraction of plasma and storage of aliquots as well as the assays used for the laboratory measurements.

Stored plasma aliquots for PRC and corticosteroids were available for the majority of patients during the follow-up visit (Table 2-2 below).

Table 2-2. Number and percentage (%) of patients with each variable measured in the overall study visit cohort (n=453).

Variable	Number of patients with each variable measured	Percentage (%) of patients with each variable Measured
PRC	445	98.2
Aldosterone	428	94.4
Cortisol	417	92
11-deoxycortisol	427	94.3

In addition to the neurohumoral and corticosteroid measurements, routine biochemical and haematological tests were undertaken during the follow-up visit. All patients had U&Es, troponin I, CRP, TSH, lipid profile, and haemoglobin measured. The assays for the aforementioned tests were described previously (section 2.3.2).

3. Chapter Three - Validation of LCMS method for plasma corticosteroid measurements

3.1 Introduction

The principal objective of the healthy volunteer studies was to validate a new method based on the LCMS technology for the assessment of plasma gluco- and mineralo-corticoid levels. The overall corticosteroid metabolite excretion rates (24-hour urine collections) rather than individual compounds have been previously assayed for the measurement of corticosteroids in large populations of patients with or without cardiovascular disease (311) (319) (320). Although urinary corticosteroid analysis provides a reliable index of average adrenal cortex activity over 24 hours, it is time-consuming and cumbersome to organise in large-scale population studies. Moreover, it is indirect as each steroid is represented by several metabolites rather than the hormone itself. Various immunoassays have been used alternatively in the research setting for the measurement of plasma corticosteroids. Although most of these assays are automated, rapid and sensitive, their specificity has been questioned due to interference by cross-reacting endogenous steroid compounds (321). In addition, immunoassays are not reliable with regards to corticosteroid analysis in normal and low concentrations (322). LCMS has become increasingly employed for plasma steroid measurements and has been characterised by its reliability properties compared with immunoassays, especially with regards to aldosterone measurements (323).

In these series of healthy volunteer studies, my aim was to ascertain that the LCMS method successfully detected predictable changes in plasma corticosteroid levels, following manipulation of the adrenal metabolism, prior to applying this method in the studies with HF patients.

3.2 Methods

3.2.1 Study design and laboratory measurements

The study design and laboratory measurements of the normal volunteer studies were described in sections 2.2.1 and 2.2.2.

3.2.2 Statistical Analysis

All statistical analyses were carried out using the Minitab 15 software. As not all variables were normally distributed, the data were log transformed before analysis. Student's paired t-test and repeated-measures analysis of variance (ANOVA) were used as appropriate to compare values between different time points. All values are presented as mean and standard deviation (SD). A value of $p<0.05$ was considered to be statistically significant.

3.3 Results

3.3.1 Visit 1−Diurnal rhythm

Cortisol and 11-deoxycortisol levels were lower at 10.00pm compared to 8.00am, 12.00pm and 4.00pm (Table 3-1). Conversely, the 11-deoxycortisol to cortisol ratio was higher in the evening, but not significantly, compared with the morning and the afternoon. A trend for lower aldosterone levels was present in the evening compared with the morning, but no differences were seen in PRC and the aldosterone to PRC ratio.

Table 3-1. Plasma corticosteroid and renin levels and 11-deoxycortisol to cortisol and aldosterone to PRC ratio at 8.00am, 12.00pm, 4.00pm and 10.00pm in visit 1

Variable	Visit 1 8.00am	Visit 1 12.00pm	Visit 1 4.00pm	Visit 1 10.00pm	p-value
Aldosterone (pmol/L)	197.8 (138.7)	136.8 (150.1)	164.5 (137.3)	124.8 (155.3)	0.302
PRC (mIU/L)	22.2 (7.1)	16.0 (5.5)	19.8 (9.0)	19.0 (7.9)	0.165
Aldosterone/PRC	9.03 (5.24)	7.48 (5.66)	8.27 (5.55)	7.06 (9.30)	0.492
11-deoxycortisol (pmol/L)	805.2 (447.3)	546.3 (369.4)	594.5 (421.4)	412.7 (608.9)	**0.004**
Cortisol (nmol/L)	378.0 (173.8)	244.4 (112.1)	215.5 (120.3)	71.7 (52.4)	**0.004**
11-deoxycortisol/cortisol (10^{-3})	2.29 (1.37)	2.21 (1.19)	3.05 (2.10)	7.24 (11.3)	0.195

Data are expressed as mean (SD)

3.3.2 Visit 2

3.3.2.1 Effects of oral glucocorticoid therapy on plasma renin and corticosteroid levels

Cortisol levels were significantly lower at baseline in visit 2 compared with visit 1 (Table 3-2 below). Correspondingly, 11-deoxycortisol levels were significantly suppressed at baseline in visit 2. Conversely, the 11-deoxycortisol to cortisol ratio was higher, but not significantly, in visit 2. Plasma aldosterone and renin concentrations and the aldosterone to PRC ratio were not significantly altered by oral glucocorticoid treatment.

Table 3-2. Plasma corticosteroid and renin levels and 11-deoxycortisol to cortisol and aldosterone to PRC ratio at baseline in visit 1 and visit 2

Variable	Visit 1 8.00am	Visit 2 8.00am	p-value
Aldosterone (pmol/L)	197.8 (138.7)	280.2 (362.8)	0.718
PRC (mIU/L)	22.2 (7.1)	26.9 (18.9)	0.980
Aldosterone/PRC	9.03 (5.24)	9.52 (10.09)	0.583
11-deoxycortisol (pmol/L)	805.2 (447.3)	277.3 (355.0)	**0.049**
Cortisol (nmol/L)	378.0 (173.8)	6.2 (5.1)	**<0.001**
11-deoxycortisol/cortisol (10^{-3})	2.29 (1.34)	82.5 (106.3)	0.130

Data are expressed as mean (SD)

3.3.2.2 Effects of ACTH on plasma renin and corticosteroid levels

A significant increase was seen in glucocorticoid levels at 30 minutes following ACTH injection (Table 3-3 below). Cortisol increased from the mean (SD) basal value of 6.2 (5.1) nmol/L to a maximum of 156.2 (53.8) nmol/L at 30 minutes and remained elevated at 60 minutes. Similarly, 11-deoxycortisol levels increased in response to ACTH and reached a maximum of 1044.7 (1036) pmol/L at 10 minutes after ACTH injection (paired t= 2.97, p= 0.021). At 30 minutes, 11-deoxycortisol levels were still higher compared with baseline. The 11-deoxycortisol to cortisol ratio was lower, but not significantly, at 30 minutes after the ACTH injection.

Table 3-3. Plasma corticosteroid and renin levels and 11-deoxycortisol to cortisol and aldosterone to PRC ratio at baseline and 30 minutes after ACTH injection in visit 2

Variable	Visit 2 0 minutes	Visit 2 30 minutes	p-value
Aldosterone (pmol/L)	280.2 (362.8)	640.8 (613.1)	**0.009**
PRC (mIU/L)	26.9 (18.9)	22.6 (12.6)	0.054
Aldosterone/PRC	9.52 (10.1)	36.9 (31.1)	**0.005**
11-deoxycortisol (pmol/L)	277.3 (355.0)	958.2 (637.8)	**0.044**
Cortisol (nmol/L)	6.2 (5.1)	156.2 (53.8)	**<0.001**
11-deoxycortisol/cortisol (10^{-3})	82.5 (106.3)	5.22 (2.79)	0.220

Data are expressed as mean (SD)

ACTH injection resulted also in a significant increase in aldosterone levels with a peak at 10 minutes (paired t= 4.94, P= 0.002). Aldosterone levels were still higher at 30 minutes compared to baseline. Conversely, there was a decrease in PRC at 30 minutes after ACTH injection although that was not statistically significant. Similar to aldosterone, the aldosterone to PRC ratio was higher at 30 minutes compared with baseline.

3.3.3 Visit 3 & 4

3.3.3.1 Effects of sodium intake on plasma renin and corticosteroid levels

Urinary sodium excretion was higher following high salt diet and lower after low salt diet (Table 3-4). Conversely, urinary potassium excretion was lower after high salt compared with lower salt intake.

The baseline plasma concentrations of gluco- and mineralo-corticoids, PRC and the 11-deoxycortisol to cortisol and aldosterone to PRC ratio are displayed in Table 3-5. Plasma aldosterone levels were higher at baseline in visit 3 (low salt diet) compared with visit 4 (high salt diet). Similarly, PRC was significantly higher at baseline after low compared to high salt diet. The shift from low to high salt diet intake suppressed aldosterone concentration in proportion to the decrease in PRC and the aldosterone to PRC ratio was not different between the two visits.

Table 3-4. Urinary electrolyte and corticosteroid excretion rates in visit 3 and 4

	Visit 3 (low salt diet)	Visit 4 (high salt diet)	p-value
Urinary sodium (mmol/L)	87.4 (32.7)	155.9 (47.5)	**0.043**
Urinary potassium (mmol/L)	79.6 (28.9)	54.2 (19.4)	**0.021**

Data are expressed as mean (SD)

No significant changes were seen in plasma glucocorticoid levels (cortisol and 11-deoxycortisol) and the 11-deoxycortisol to cortisol ratio in response to salt diet manipulation.

Table 3-5. Plasma corticosteroid and renin levels and 11-deoxycortisol to cortisol and aldosterone to PRC ratio at baseline in visit 3 and 4

Variable	Visit 3 (low salt diet) 0 minutes	Visit 4 (high salt diet) 0 minutes	p-value
Aldosterone (pmol/L)	194.2 (127.6)	72.1 (66.6)	0.053
PRC (mIU/L)	42.9 (30.2)	11.2 (5.9)	**<0.001**
Aldosterone/ PRC	7.20 (9.82)	8.99 (11.39)	0.572
11-deoxycortisol (pmol/L)	467.5 (303.0)	718.6 (476.2)	0.222
Cortisol (nmol/L)	251.1 (143.5)	251.1 (193.1)	0.534
11-deoxycortisol/cortisol (10^{-3})	1.83 (1.13)	3.4 (2.65)	0.178

Data are expressed as mean (SD)

3.3.3.2 Effects of angiotensin II on blood pressure

During visit 4, angiotensin II infusion was discontinued in one normal volunteer, 35 minutes from the baseline, as there was a more than 20 mmHg increase in the MAP.

During angiotensin II infusion, a slight, but significant, increase in DBP and MAP occurred at both visits 3 and 4 confirming the biological activity of the peptide (Table 3-6). The DBP increased from the basal level of 69 (7) mmHg to 74 (9) mmHg at 60 minutes in Visit 3 and from 69 (3) mmHg to 77 (5) mmHg at 60 minutes in Visit 4. Similarly, the MAP increased from 80 (7) mmHg at baseline to 85 (8) mmHg at 60 minutes in Visit 3 and from 80 (2) mmHg to 88 (6) mmHg at 60 minutes in Visit 4. Correspondingly, the SBP increased from baseline in both visits 3 and 4 but that reached statistical significance only at 60 minutes in Visit 4.

Table 3-6. Blood pressure measurements at 0 minutes and 60 minutes after angiotensin II infusion in visit 3 and 4

Variable	Visit 3 (low salt diet)			Visit 4 (high salt diet)		
	0 minutes	**60 minutes**	**p-value**	**0 minutes**	**60 minutes**	**p-value**
SBP (mmHg)	110 (9)	112 (10)	0.195	110 (9)	118 (2)	**0.033**
DBP (mmHg)	69 (7)	74 (9)	**0.005**	69 (3)	77 (5)	**0.014**
MAP (mmHg)	80 (7)	85 (8)	**0.002**	80 (2)	88 (6)	**0.014**

Data are expressed as mean (SD)

3.3.3.3 Effects of angiotensin II on plasma renin and corticosteroid levels

Angiotensin II infusion induced a significant increase in aldosterone secretion at 60 minutes compared with baseline in visit 3 and 4 (Table 3-7). Conversely, infusion of angiotensin II resulted in a decrease of PRC at 60 minutes in both visit 3 and 4. Plasma cortisol levels were significantly lower at 60 minutes compared with baseline in both visits. No significant differences were observed in 11-deoxycortisol levels or the 11-deoxycortisol to cortisol ratio between 60 minutes and baseline in visit 3 and 4.

Table 3-7. Plasma corticosteroid and renin levels and 11-deoxycortisol to cortisol and aldosterone to PRC ratio at 0 minutes and 60 minutes after angiotensin II infusion in visit 3 and 4

Variable	Visit 3 (low salt diet)			Visit 4 (high salt diet)		
	0 min	**60 min**	**p-value**	**0 min**	**60 min**	**p-value**
Aldosterone (pmol/L)	194.2 (127.6)	776.7 (574.6)	**0.005**	72.1 (66.6)	524.3 (521.5)	**0.001**
PRC (mIU/L)	42.9 (30.2)	18.8 (14.6)	**0.002**	11.2 (5.9)	8.0 (4.0)	**0.029**
Aldosterone/PRC	7.20 (9.8)	48.72 (22)	**<0.001**	8.99 (11.39)	71.1 (61.3)	**0.001**
11-deoxycortisol (pmol/L)	467.5 (303.0)	415.6 (346.3)	0.505	718.6 (476.2)	421.4 (447.3)	0.052
Cortisol (nmol/L)	251.1 (143.5)	162.8 (138)	**0.004**	251.1 (193.1)	157.3 (179.3)	**<0.001**
11-deoxycortisol/cortisol (10^{-3})	1.83 (1.13)	2.86 (2.12)	0.213	3.4 (2.65)	4.74 (6.4)	0.458

Data are expressed as mean (SD)

3.3.3.4 Effects of sodium intake on aldosterone responsiveness to angiotensin II

Following low sodium diet in visit 3, angiotensin II increased plasma aldosterone by 79.3 (163.4), 464.9 (372.8) and 582.5 (533.4) pmol/L at the incremental infusion rates of 1, 3 and 5ng/kg/min respectively (Table 3-8 below). On the other hand, after high salt diet, angiotensin II produced an increase in aldosterone by 56.4 (76.8), 208.6 (152) and 450.8 (527.1) pmol/L at the three incremental infusion rates. The increments of aldosterone were significantly greater after low than after high salt diet at the two higher angiotensin II infusion rates (3 & 5 ng/kg/min). No significant difference in aldosterone increment was evident at the lower angiotensin infusion rate (1ng/kg/min) between the two visits

Table 3-8. Increments of plasma aldosterone levels at 20, 40 and 60 minutes from baseline in visit 3 and 4

Angiotensin II infusion rate	Visit 3 (low salt)	Visit 4 (high salt)	p-value
	Increments of aldosterone from baseline (pmol/L)	Increments of aldosterone from baseline (pmol/L)	
20 min (1ng/kg/min)	79.3 (163.4)	56.4 (76.8)	0.367
40 min (3ng/kg/min)	464.9 (372.8)	208.6 (152)	**0.043**
60 min (5ng/kg/min)	582.5 (533.4)	450.8 (527.1)	**0.041**

Data are expressed as mean (SD)

3.4 Discussion

In these series of healthy volunteer studies, the LCMS method effectively detected changes in plasma steroid levels, predictable from many previous studies, following manipulation of adrenal metabolism.

3.4.1 Visit 1 & 2 – Diurnal rhythm, effects of oral glucocorticoid treatment and ACTH injection on plasma corticosteroid and renin levels

In visit 1, a diurnal pattern in glucocorticoid secretion was clearly demonstrated in this small cohort of healthy subjects. The ACTH-dependent adrenal glucocorticoids (11-deoxycortisol and cortisol) were higher in the morning than in the afternoon and in the evening due to the greater release of ACTH by pituitary gland in the morning. ACTH stimulates cholesterol entry into mitochondria, increases the conversion of cholesterol to pregnenolone and up-regulates the expression of the enzymes involved in the glucocorticoid pathway (section 1.2.4). Conversely, there was a trend for lower 11-deoxycortisol to cortisol ratio, which is an index of 11beta-hydroxylase activity, in the morning, reflecting a higher enzyme activity, as a higher concentration of the end product is secreted into the circulation compared with the substrate used.

Apart from the glucocorticoid levels, the circadian pattern was also evident in the secretion of mineralocorticoids, with aldosterone reaching minimum levels late in the evening, although the differences were not significant. Aldosterone secretion has been shown to exhibit a circadian variation with increasing levels in the morning and lower levels in the evening, due to the diurnal pattern of ACTH secretion (324) (325).

Treatment with an oral glucocorticoid resulted, as expected, in a reduction of plasma glucocorticoid levels at baseline in visit 2 compared with visit 1. Dexamethasone suppresses

the endogenous ACTH production via a negative feedback circuit and results in a decrease of plasma glucocorticoid levels. Indeed, that was evident for both 11-deoxycortisol and cortisol levels. For each of the two glucocorticoids (11-deoxycortisol and cortisol) studied, significant increases in their plasma levels were seen in response to low dose of ACTH in visit 2. This is expected for adrenal steroids produced in ZF, which is under the ACTH control, and is in agreement with findings from early experiments with ACTH infusions in healthy subjects injected with standard (250μg) or low (1μg) dose of ACTH (326) (327) (328).

Apart from the increase in glucocorticoid levels, an increase in plasma aldosterone concentration was evident in response to low ACTH dose in visit 2. That is consistent with the ACTH regulatory influence on the ZG cells in the short term (327) (329).

3.4.2 Visit 3 & 4 – Effects of sodium intake and angiotensin II on plasma corticosteroid and renin levels

A higher PRC and aldosterone concentration was manifested at baseline after low compared with high salt diet (Visit 3 versus Visit 4) in these studies. Sodium concentration at the renal distal tubule is one of the principal regulators of renin release by the juxta-glomerular apparatus in the kidneys and dietary sodium deprivation in man is followed by enhanced activity of the RAAS (section 1.2.3). Apart from the effect on renin secretion, sodium restriction has additionally been shown to directly increase the activity of aldosterone synthase and thereby the secretion of aldosterone (103) (330). In the Framingham offspring sub-study, urinary sodium, which is a measure of sodium intake, was the strongest independent determinant of serum aldosterone levels (331).

With regards to the glucocorticoid secretion, no differences were found in 11-deoxycortisol and cortisol at baseline in visits 3 and 4. This is expected, as ZF is not sensitive to

extracellular sodium levels and the enzymes involved in the synthesis of glucocorticoids are principally controlled by ACTH.

Angiotensin II induced a significant increase in aldosterone release in both sodium-replete and sodium-deplete healthy subjects. Acute response to angiotensin II involves an increase of the conversion of cholesterol and other precursors to aldosterone (section 1.2.3). In contrast to aldosterone stimulation, angiotensin II suppressed PRC in both visit 3 and 4. The inhibitory effect of angiotensin on renin secretion represents a negative feedback mechanism; angiotensin directly suppresses renin release by JGA through a feedback loop, independent of changes in blood pressure, exerting in this way an auto-regulatory control on its own activation.

Plasma cortisol concentrations did not rise, as expected, in response to angiotensin infusion in both visit 3 & 4. In contrast, plasma cortisol and 11-deoxycortisol levels fell during angiotensin infusion and that is likely to represent a decline due to circadian rhythm. In agreement with this finding, cortisol levels in visit 1 were significantly lower at 10.00am compared with 8.00am in the same visit.

Finally, during angiotensin infusion there was a significant difference in the increments of aldosterone in response to increasing infusion rate between the two visits. Normal subjects responded to angiotensin, especially in the higher infusion rates, with greater increase in aldosterone levels after low compared with high salt diet. This is in agreement with previous studies in humans; the slope of aldosterone to AII regression curve was steeper in healthy subjects infused with angiotensin II after low than after high salt diet (83) (84).

Overall, in this chapter, I successfully produced physiological responses of endogenous corticosteroids, which were analysed by the LCMS method, following manipulation of the HPA axis and RAAS. These studies confirmed the reliability of the LCMS method for corticosteroid measurements prior to application in the studies with HF patients.

4. Chapter Four - Baseline patient characteristics during hospital admission

4.1 Introduction

The main purpose of this chapter is to describe the baseline characteristics in patients admitted to hospital with decompensated HF. These characteristics include demographic data, medical history, physiological measurements, 12-lead ECG findings and transthoracic echocardiogram parameters, laboratory measurements and medication prior to hospital admission. In this chapter, I also show the plasma levels of RAAS components and glucocorticoids in these patients. The markers of RAAS activity measured were PRC and plasma aldosterone. The glucocorticoids measured were plasma 11-deoxycortisol and cortisol. In addition, the aldosterone to PRC ratio and the 11-deoxycortisol to cortisol ratio were calculated. Lastly, I present the levels of RAAS components and glucocorticoids according to background therapy with a RAAS inhibitor and an oral glucocorticoid respectively.

4.2 Methods

4.2.1 Study design and laboratory measurements

Details of the study participants and the laboratory measurements were presented previously in sections 2.3.1 & 2.3.2.

4.2.2 Statistical analysis

All baseline characteristics were expressed as median (interquartile range, IQR) for continuous and as absolute number (percentage) for categorical variables. The inter-group comparisons were carried out by the Mann-Whitney and Kruskal-Wallis test for continuous variables as appropriate and by the χ^2 test for categorical variables. A p-value <0.05 was considered significant for all analyses. Statistical analyses were performed with Minitab version 15.

4.3 Results

4.3.1 Baseline patient characteristics during hospital admission

A total of 1631 patients with suspected HF were screened for inclusion in the study and 909 of them were excluded (Figure 4.1).

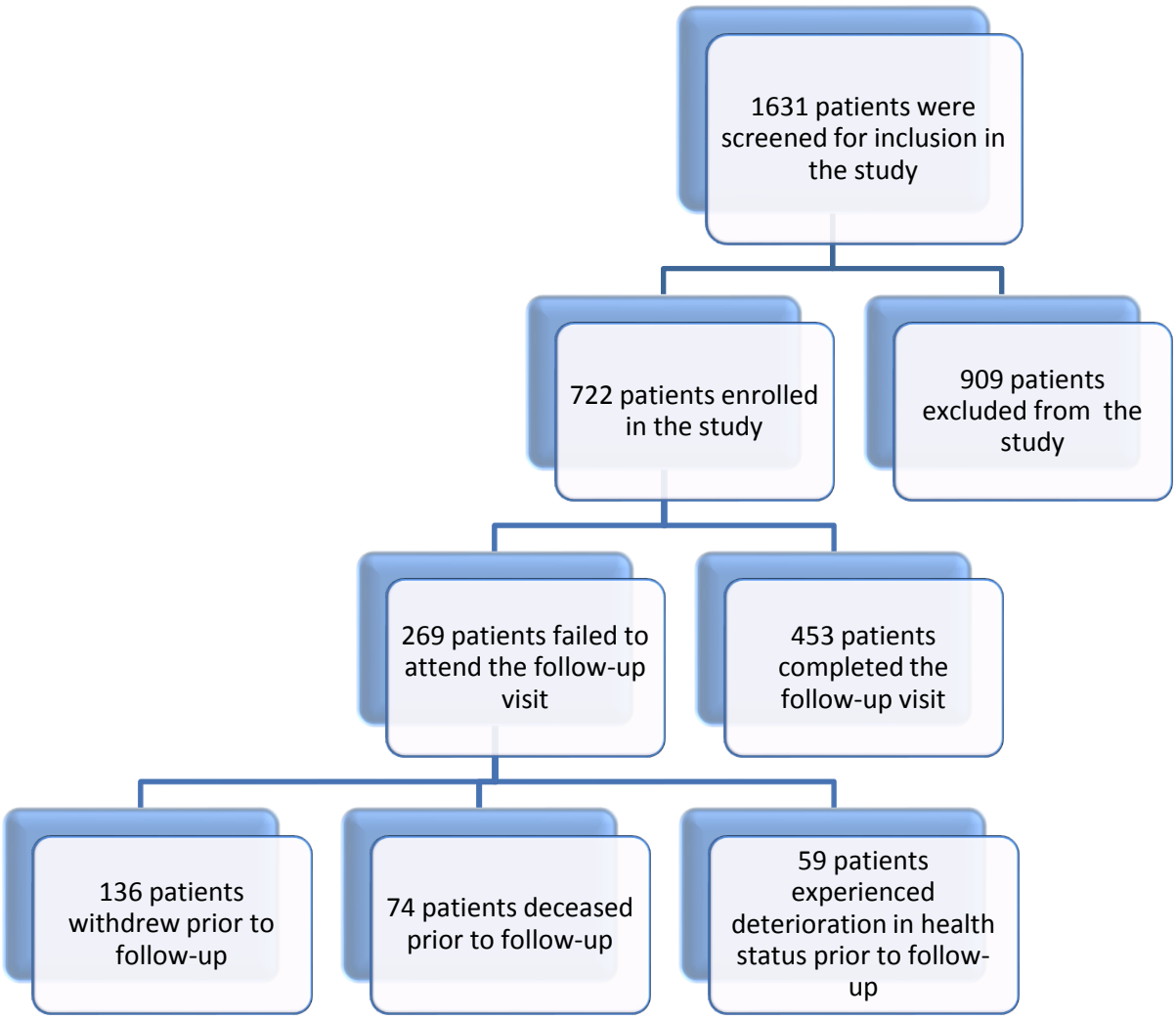


Figure 4-1. Flow of patients through the study

The breakdown of reasons for exclusion from participation in the study is presented in Figure 4.2.

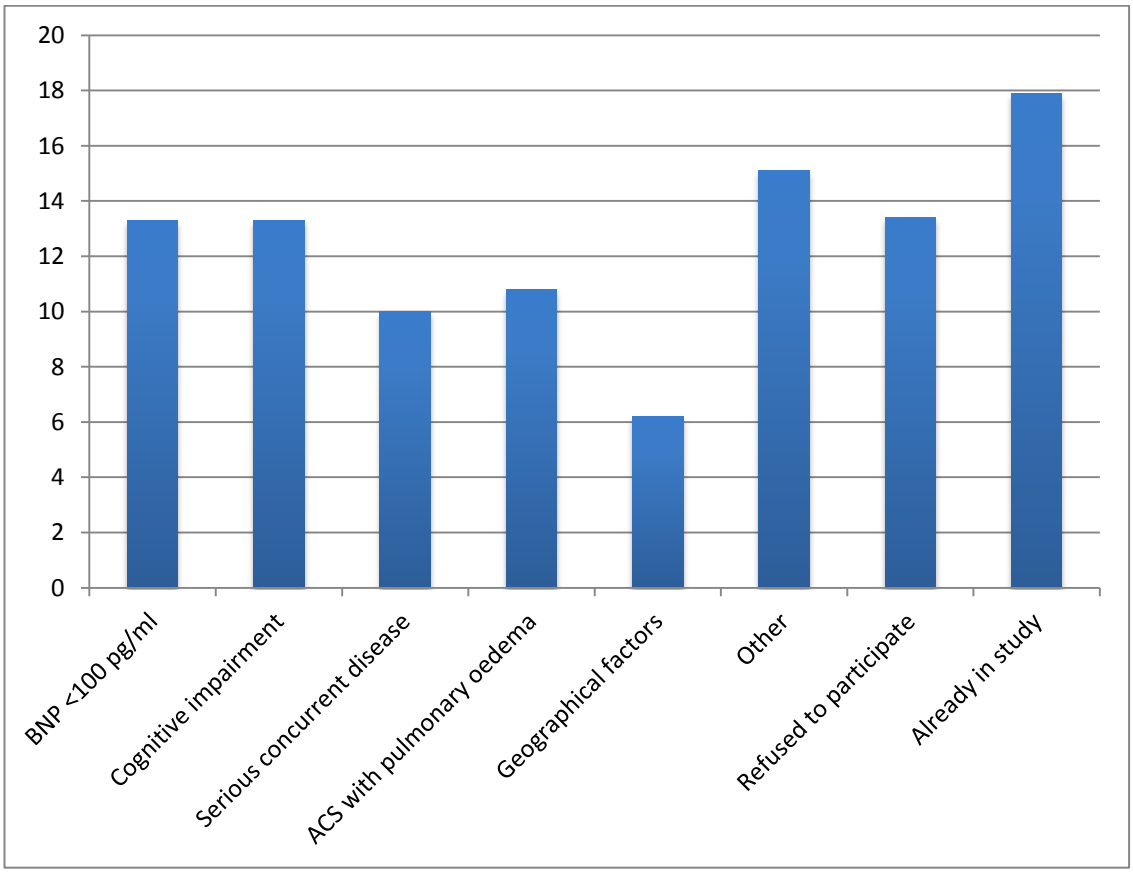


Figure 4-2. Reasons for exclusion from participation into the study

The most common reason was re-admission with HF, reflecting the high rate of rehospitalisation due to HF in these patients. The second most common single reason was refusal to participate. The other more common reasons for exclusion from participation were BNP <100 pg/ml and cognitive impairment. Moreover, patients who required ambulance for transfer to the BHF Glasgow Cardiovascular Research Centre for the follow-up visit (nursing home residents and homebound patients - under "Other" in Figure 4.2) were also excluded from the study. Finally, patients with BNP≥100 pg/ml but with alternative diagnosis, such as exacerbation of chronic obstructive pulmonary disease or pulmonary embolism who

responded to treatment for the above conditions, were also excluded (under "Other" in Figure 4.2).

722 patients with decompensated HF were enrolled in the study. The baseline characteristics of the hospitalised cohort are presented in Table 4-1. The median age (IQR) of the study participants was 74 (68 - 81) years and forty-six percent were women. Approximately three quarters of patients during hospital admission, were in NYHA functional class III (60.2%) and IV (15.7%) and one quarter (24.1%) of patients were in NYHA II class. A previous history of HF was present in forty-four percent of patients and a similar proportion had a history of previous MI. Sixty-six percent of patients had pre-existing hypertension and thirty-one percent had history of diabetes. While just over half of patients (53.6%) had a history of AF, only forty-one percent had this arrhythmia on their screening ECG.

The median (IQR) BMI was 27.9 (24 - 32.9) kg/m^2. Twenty-nine percent of patients were overweight (defined as BMI 25-30 kg/m^2) and thirty-nine percent were obese (BMI $\geq$30.kg/m^2). A small proportion of patients (2.6%) were underweight during admission (BMI <18.5kg/m^2).

The median (IQR) SBP was 134 (115 - 152) mmHg. More than a third (40.7%) of patients had SBP ≥ 140mmHg with more than half (50.4%) of these patients having isolated systolic hypertension (SBP ≥ 140mmHg and DBP <90 mmHg). A small proportion (1.8%) of patients presented with SBP <90 mmHg. Approximately twenty percent of patients had DBP > 90mmHg and a similar proportion presented with DBP <60mmHg on admission. The median (IQR) pulse rate was 86 (71.8 - 106) beats per minute (bpm). One third of patients were tachycardic (pulse rate >100bpm) while less than ten percent were bradycardic (pulse rate <60bpm).

Two thirds of patients had LVSD, while more than a third (37.3) had dilated left ventricle and less than half (44%) had LVH on the transthoracic echocardiogram.

The median BNP (IQR) of the hospitalised cohort was raised at 871pg/ml (391 -1819). Sodium levels with a median of 138 mmol/L were at the lower normal levels. A higher proportion of patients had hyponatraemia (sodium <135 mmol/L) than hypernatraemia (sodium >145 mmol/L) during admission (19% vs 3.7%). The median eGFR was reduced at 56 ml/min/1.73m^2. More than half of the patients (56.1%) had an eGFR <60 ml/min/1.73m^2 and approximately a tenth (10.9 %) had an eGFR <30 ml/min/1.73m^2. The median creatinine (106.5 μmol/L) was within the normal range. Despite this, the median urea (8.7 mmol/L) was raised.

Of the 620 patients recruited at the Western and Royal Infirmaries, the majority (96.6%) had troponin measurements available during admission. Elevated troponin (defined as troponin I ≥ 0.04 μg/L) was present in more than half of patients (53.2%). From the 102 patients recruited at the RAH, only a third had troponin measured and this was elevated (defined as

troponin T ≥ 0.05 μg/L) in less than a quarter of these patients. The median CRP was raised at 13 mg/L with more than half of the patients (59.7%) having a CRP > 10 mg/L. TSH and lipids were generally within normal range.

The median haemoglobin was 12.1g/dl with a minimum of 6 g/dl and maximum of 18.6 g/dl. More than half of males (56.9%) and females (55.1%) had anaemia, defined as haemoglobin <13g/dl and haemoglobin <12g/dl respectively (332).

Just over two thirds of patients (69%) were taking an oral diuretic prior to hospital admission. Almost sixty percent (59.6%) were taking either an ACE inhibitor or ARB, while approximately half of patients (49.6%) were taking an ACE inhibitor prior to admission. A similar proportion of patients (47.9%) were taking a beta-blocker, while less than a tenth of patients (8.3%) were treated with an aldosterone antagonist. Only a small proportion of patients (3.6%) were receiving oral glucocorticoid therapy.

The majority of patients (99.1%) were treated with IV or oral diuretic during the first 24 hours following hospital admission. Other treatments given during admission are shown in Table 4-1.

Table 4-1. Baseline patient (n=722) characteristics during hospital admission

Variable	Median or number of patients	Interquartile range (IQR) or %
Age (years)	74	68 – 81
Female gender	332	46
NYHA class		
II	174	24.1
III	435	60.3
IV	113	15.7
Medical history		
HF	320	44.3
MI	322	44.6
Angina	396	54.8
Diabetes mellitus	227	31.4
Hypertension	478	66.2
AF	387	53.6
CVA/TIA	155	21.5
Physiological measurements		
BMI (kg/m^2)	27.9	24 - 32.9
Weight (kg)	76	60 - 90
Pulse rate (bpm)	86	71.8 - 106
SBP (mmHg)	134	115 - 152
DBP (mmHg)	75	62 - 88
Signs of fluid congestion		
Elevated JVP	512	79.4
Peripheral oedema	542	75.1
ECG rhythm		
SR	398	55.1
AF	294	40.7
Echocardiogram measurements		
LVEDD (cm)	5.2	4.6 - 5.9
Dilated left ventricle	191	37.3
LVH	226	44
LVSD	341	66.6
Laboratory measurements (blood)		
BNP (pg/ml)	871	391 - 1819
Troponin I ≥ 0.04 (µg/L)*	330	53.2
Sodium (mmol/L)	138	135 - 141

Variable	Median or number of patients	Interquartile range (IQR) or %
Potassium (mmol/L)	4.2	3.8 - 4.5
Urea (mmol/L)	8.7	6.3 - 12
Creatinine (µmol/L)	106.5	85 - 137
eGFR (ml/min/1.73m^2)	56	41- 60
eGFR <60ml/min/1.73m^2	405	56.1
Cholesterol (total) (mmol/L)	3.7	3.1- 4.6
HDL (mmol/L)	1.0	0.8 - 1.3
CRP (mg/L)	13	5.7 - 32
TSH (mIU/L)	1.7	1.0 - 2.8
Haemoglobin (g/dl)	12.1	10.6 - 13.5
Cardiovascular medication prior to admission		
Diuretic	498	69
Furosemide	421	58.3
ACE inhibitor	358	49.6
ACE inhibitor or ARB	430	59.6
Aldosterone blocker	49	6.8
Beta-blocker	346	47.9
Digoxin	117	16.2
Anti-arrhythmic	29	4.0
Aspirin	388	53.7
Statin	471	65.2
Non-cardiovascular medication prior to admission		
Steroid tablets	26	3.6
Cardiovascular medication during admission		
Diuretic	716	99.1
IV nitrate	68	9.4
IV inotropes	16	2.2

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)

Abbreviations: NYHA, New York Heart Association; HF, Heart Failure; MI, myocardial infarction; AF, atrial fibrillation; CVA, cerebrovascular accident; TIA, transient ischaemic attack; BMI, body mass index; bpm, beats per minute; SBP, systolic blood pressure; DBP, diastolic blood pressure; SR, sinus rhythm; LVEDD, left ventricular end-diastolic diameter; LVH, left ventricular hypertrophy; LVSD, left ventricular systolic dysfunction; BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; CRP, C-reactive protein; TSH, thyroid stimulating hormone; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker IV, intravenous

***** measured at WIG and GRI

4.3.2 Baseline levels of RAAS components during hospital admission

Levels of plasma aldosterone and PRC and the aldosterone to PRC ratio during hospital admission are presented below.

4.3.2.1 Aldosterone

An aldosterone level measured during hospital admission was available in 551 of the 722 patients. The median (IQR) aldosterone was 72.3 (31.7 – 151.6) pmol/L (Figure 4-3) and the mean (SD) aldosterone was 141 (289) pmol/L. A frequency distribution histogram of aldosterone levels during the hospital admission in these patients is displayed in Figure 4-4. The distribution of aldosterone concentrations was positively skewed. The minimum aldosterone concentration was 0.8 pmol/L and the maximum aldosterone concentration was 5398.8 pmol/L. The normal range for aldosterone measured in a pool of normal plasma samples by LCMS in the BHF Glasgow Cardiovascular Research Centre laboratory was 0 – 937 pmol/L. The majority of patients (98.9%) had aldosterone levels within the normal range and only six patients (1.1%) had aldosterone levels above the upper limit of normal. Review of the past medical history (that was documented for every patient enrolled in the study during recruitment) in patients with aldosterone levels above the normal range revealed the presence of Conn's syndrome in one of these patients (patient with aldosterone concentration of 1045.2 pmol/l). PRC was at the lowest detectable level (5.0 mIU/L) and aldosterone to PRC ratio was markedly elevated (209.1) in that patient (see section 4.3.2.3). In all the other patients with aldosterone values higher than the upper limit of normal, PRC values were elevated (180.1 to 4898 mIU/L) resulting in aldosterone to PRC ratio ≤9.0.

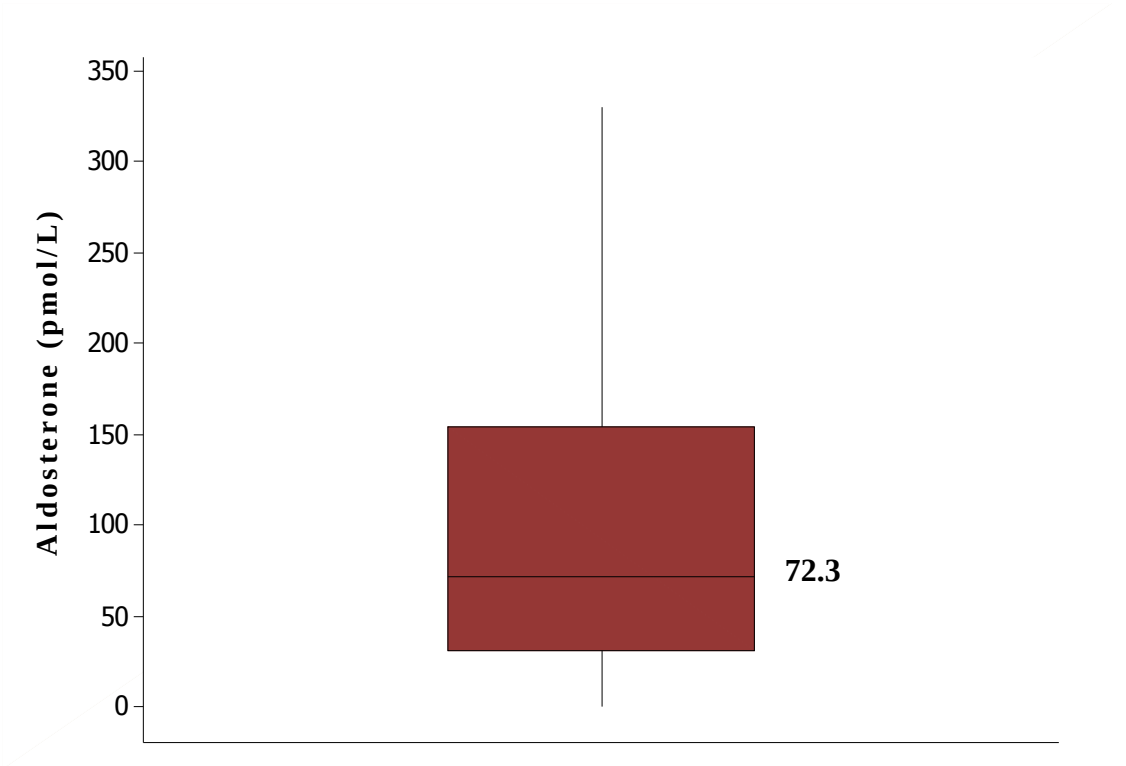


Figure 4-3. Box and whisker plot of the aldosterone concentrations showing the 2.5, 25, 50, 75 and 97.5 centiles

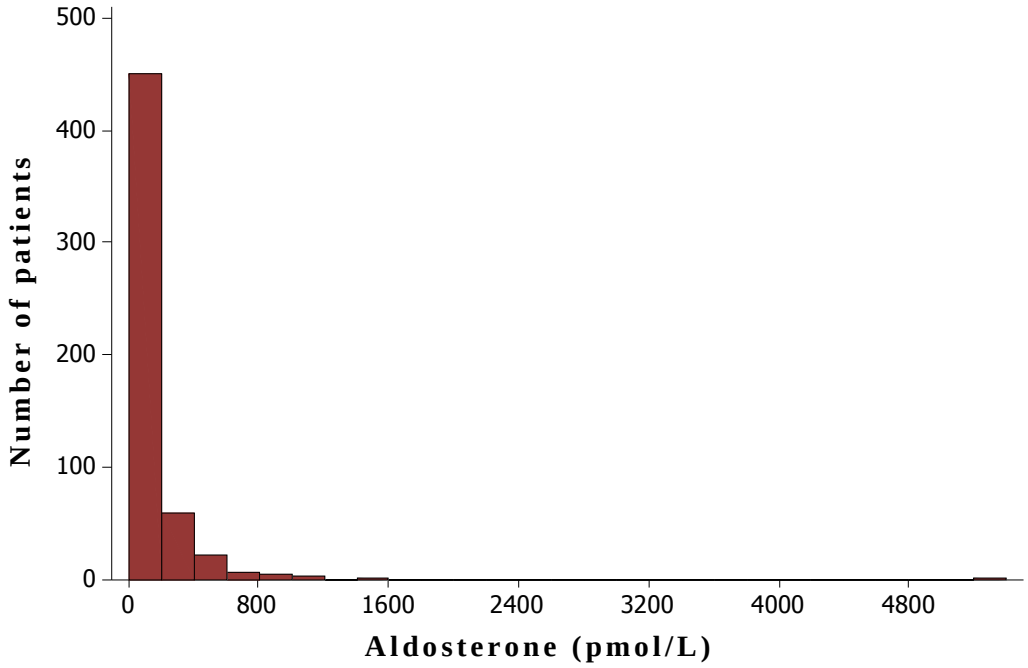


Figure 4-4. Frequency distribution histogram of aldosterone concentrations in patients during hospital admission

4.3.2.2 PRC

A PRC measured during hospital admission was available in 689 of the 722 patients. The median (IQR) PRC was 47.3 (13.0 - 177.3) mIU/L (Figure 4-5) and the mean (SD) PRC was 408 (1268) mIU/L. A frequency distribution histogram of PRC levels during the hospital admission in these patients is displayed in Figure 4-6. The distribution of PRC levels was positively skewed. The minimum PRC was 5.0 mIU/L and the maximum PRC was 12668 mIU/L. The normal range for PRC analysed by the Diasorin direct assay is 5.0 - 44.9 mIU/L (305). Approximately half of patients (48.4%) had PRC within the normal range.

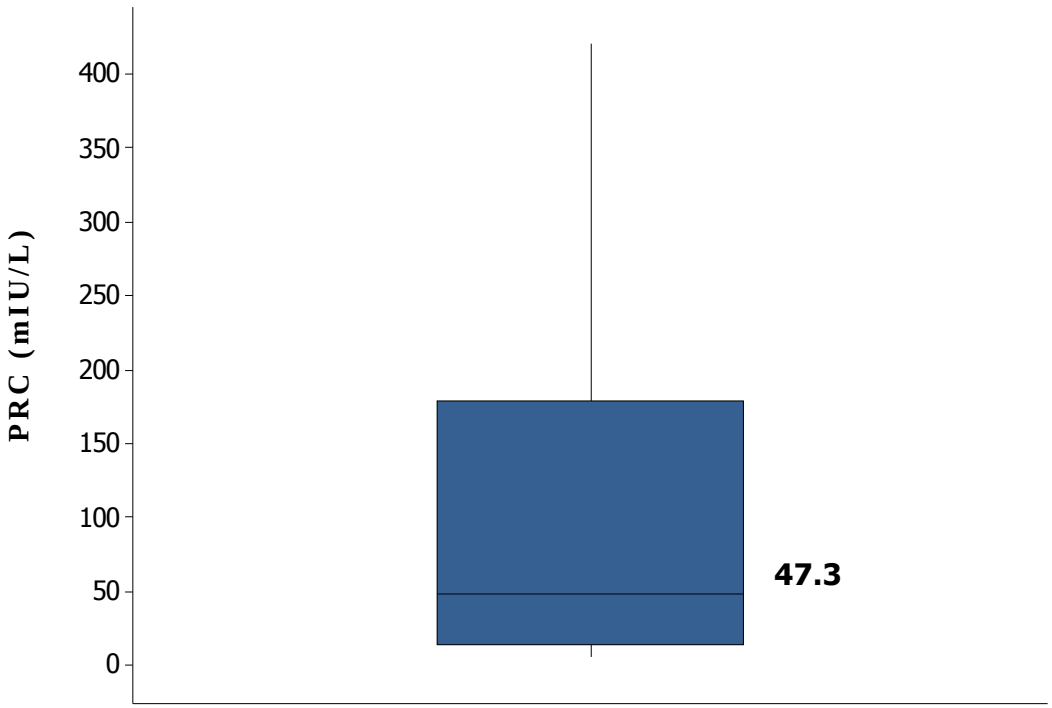


Figure 4-5. Box and whisker plot of the PRC values showing the 2.5, 25, 50, 75 and 97.5 centiles

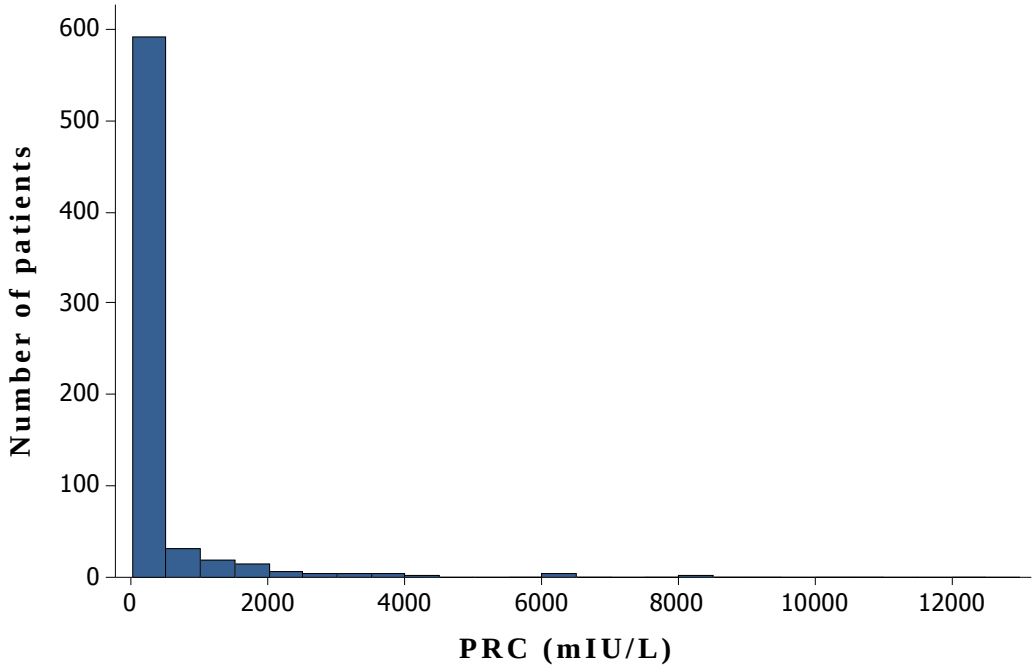


Figure 4-6. Frequency distribution histogram of PRC levels in patients during hospital admission

4.3.2.3 Aldosterone to PRC ratio

An aldosterone to PRC ratio could be calculated for 542 of the 722 patients studied. The median (IQR) aldosterone to PRC ratio was 1.53 (0.30-4.95) (Figure 4-7) and the mean (SD) aldosterone to PRC was 5.3 (14.4). A frequency distribution histogram of aldosterone to PRC ratio during hospital admission in these patients is displayed in Figure 4-8. The distribution of aldosterone to PRC values was positively skewed. The minimum aldosterone to PRC ratio was 0.001 and the maximum aldosterone to PRC ratio was 209.1. The highest aldosterone to PRC ratio calculated was found in the patient with history of Conn's syndrome. No other patient in the group of extreme outliers with high aldosterone to PRC ratio values (aldosterone to PRC ratio value > 60) was found to have history of Conn's syndrome or primary aldosteronism. These patients had PRC of 5mIU/L and aldosterone levels between 478pmol/L and 784pmol/L.

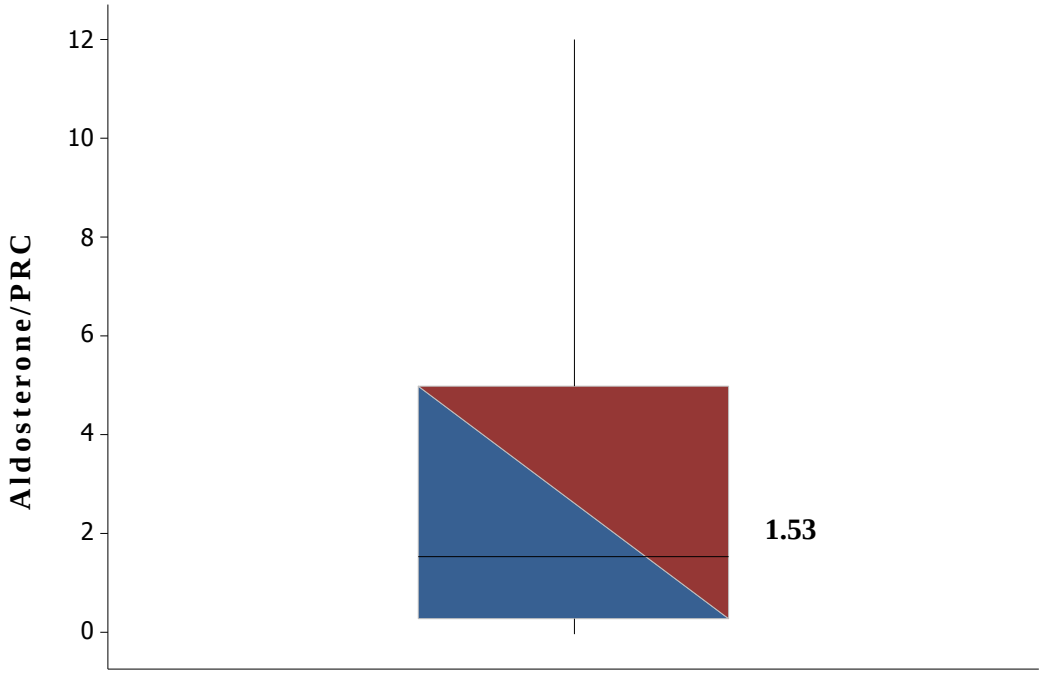


Figure 4-7. Box and whisker plot of the aldosterone to PRC ratio showing the 2.5, 25, 50, 75 and 97.5 centiles

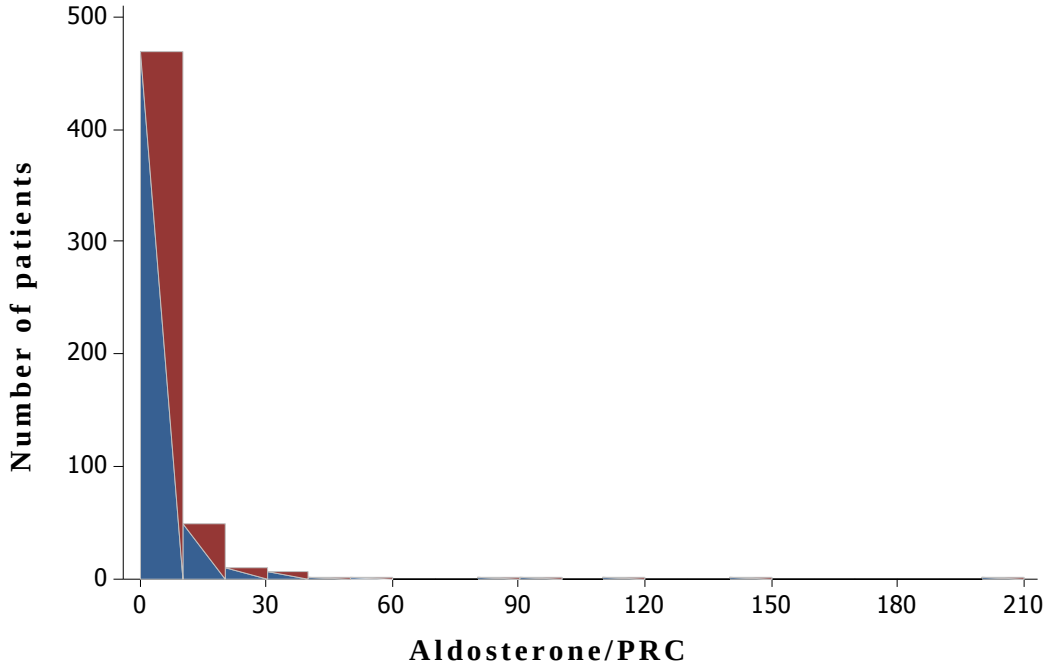


Figure 4-8. Frequency distribution histogram of aldosterone to PRC ratio in patients during hospital admission

4.3.2.4 Baseline levels of RAAS components according to background therapy with a RAAS inhibitor

Levels of the RAAS mediators were stratified according to background therapy with an ACE inhibitor/ARB or aldosterone blocker (Table 4-2). Plasma aldosterone at baseline was higher in patients taking an aldosterone blocker but not an ACE inhibitor or ARB and lower in patients taking an ACE inhibitor or ARB but not an aldosterone blocker prior to admission (Table 4-2). PRC was higher in patients taking an ACE inhibitor/ARB or aldosterone blocker and lower in patients taking neither an ACE inhibitor/ARB nor an aldosterone blocker prior to admission. Conversely, aldosterone to PRC ratio was lower in patients taking a RAAS inhibitor and higher in patients not taking a RAAS inhibitor.

Table 4-2. Levels of RAAS mediators during hospital admission according to background therapy with an ACE inhibitor/ARB or aldosterone blocker

Variable	All patients (n=722)	ACE inhibitor/ARB (No) Aldosterone blocker (No) (n=278)	ACE inhibitor/ARB (Yes) Aldosterone blocker (No) (n=395)	ACE inhibitor/ARB (No) Aldosterone blocker (Yes) (n=14)	ACE inhibitor/ARB (Yes) Aldosterone blocker (Yes) (n=35)	p-value†
Aldosterone (pmol/L)	72.3 (31.7 - 151.6)	80.3 (41.8 - 184.7)	63.4(26.1 - 128.7)	217.3 (96.7 - 344.8)	82.4 (24.3 -199.7)	**<0.001**
PRC (mIU/L)	47.3 (13.0 - 177.3)	27.2 (5.0 - 78.6)	66.3 (16.2 - 309.8)	310 (38 - 510)	236 (64 - 1752)	**<0.001**
Aldosterone/ PRC	1.53 (0.30 – 4.95)	3.11 (1.09 - 7.51)	0.83(0.180 - 3.56)	1.53 (0.64 - .84)	0.23(0.06 - 1.30)	**<0.001**

Values are presented as median (IQR)

† Kruskal-Wallis test was used for inter-group comparisons

4.3.3 Baseline glucocorticoid levels during hospital admission

Levels of plasma 11-deoxycortisol and cortisol and the 11-deoxycortisol to cortisol ratio during hospital admission are presented below.

4.3.3.1 11-deoxycortisol

An 11-deoxycortisol level measured during hospital admission was available in 600 of 722 patients. The median (IQR) 11-deoxycortisol was 492.6 (275.5 – 929.6) pmol/L (Figure 4-9) and the mean (SD) 11-deoxycortisol was 917 (1716) pmol/L. A frequency distribution histogram of 11-deoxycortisol levels during the hospital admission in these patients is displayed in Figure 4-10. The minimum 11-deoxycortisol concentration was 3.7 pmol/L and the maximum 11-deoxycortisol concentration was 21666 pmol/L. The normal range for 11-deoxycortisol analysed by LCMS is 0 - 2017 pmol/L. The majority of patients (91%) had 11-deoxycortisol levels within the normal range.

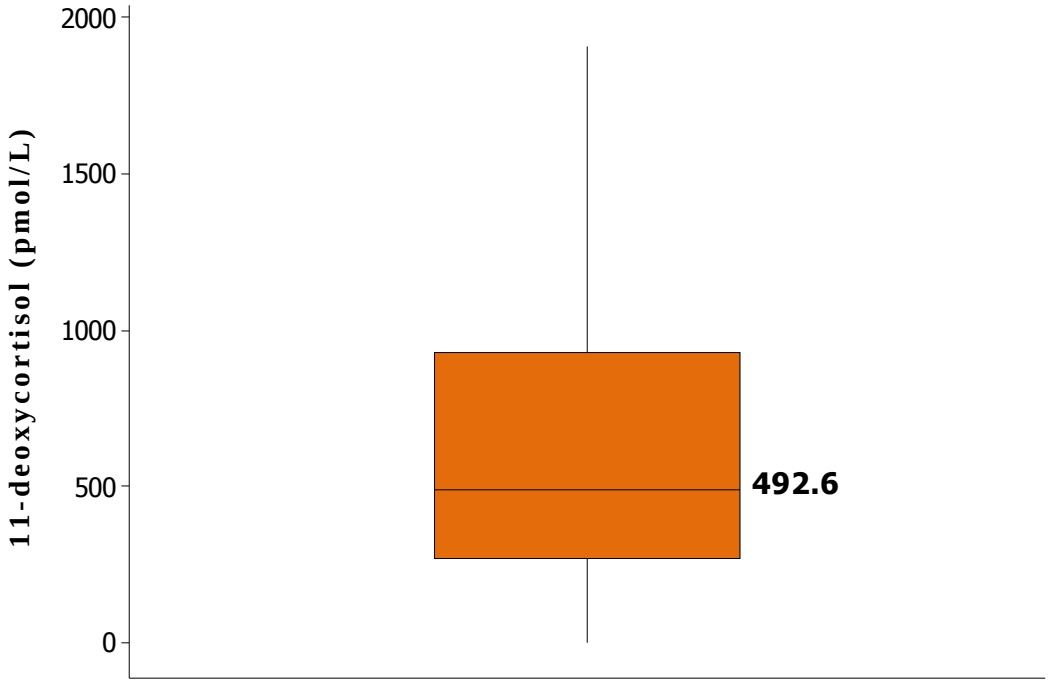


Figure 4-9. Box and whisker plot of 11-deoxycortisol showing the 2.5, 25, 50, 75 and 97.5 centiles

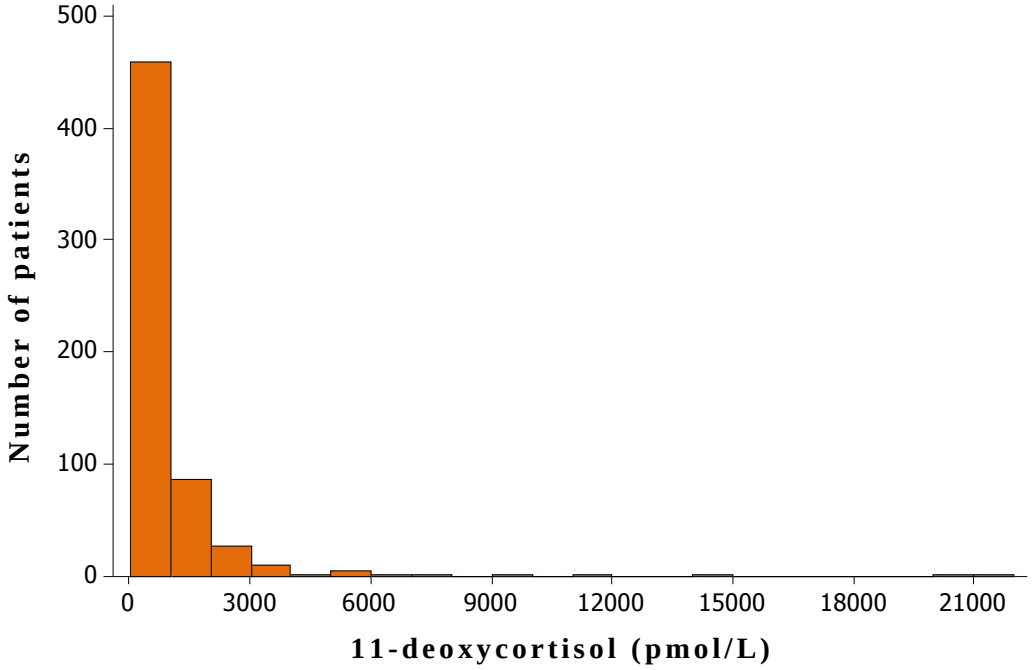


Figure 4-10. Frequency distribution histogram of 11-deoxycortisol levels in patients during hospital admission

4.3.3.2 Cortisol

A cortisol level measured during hospital admission was available in 613 of 722 patients. The median (IQR) cortisol was 322.6 (226.0 - 444.4) nmol/L (Figure 4.11) and the mean (SD) cortisol was 358 (231) nmol/L. A frequency distribution histogram of cortisol levels during the hospital admission in these patients is displayed in Figure 4-12. The minimum cortisol concentration was 1.7 nmol/L and the maximum cortisol concentration was 3123.2 nmol/L. The normal range for cortisol analysed by LCMS is 0 - 823nmol/L. The majority of patients (98%) had cortisol levels within the normal range.

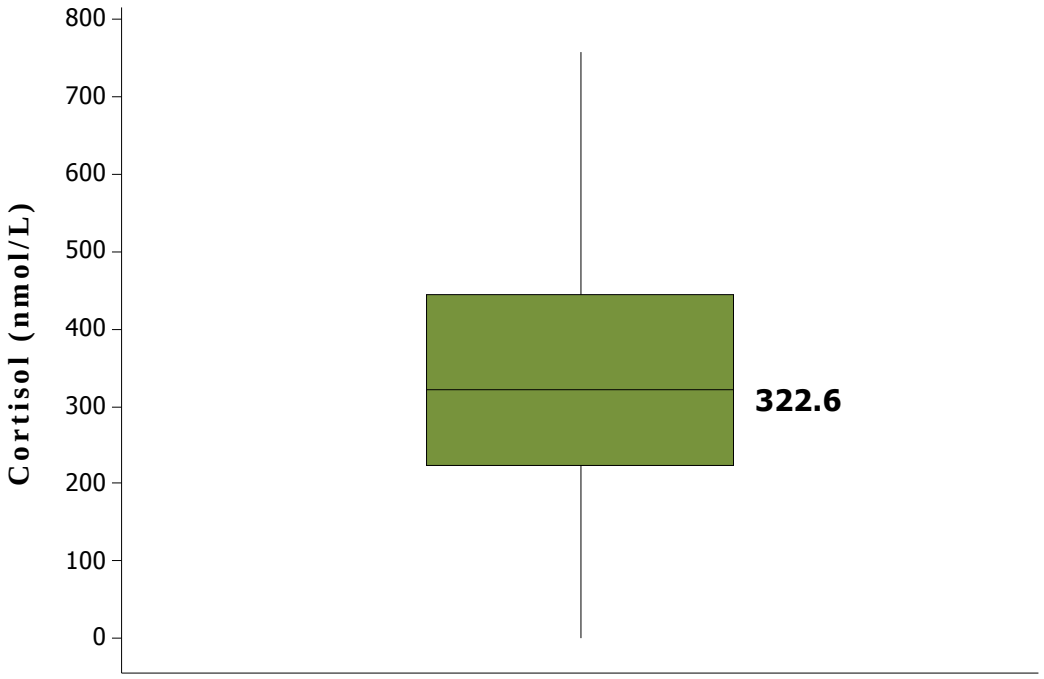


Figure 4-11. Box and whisker plot of cortisol showing the 2.5, 25, 50, 75 and 97.5 centiles

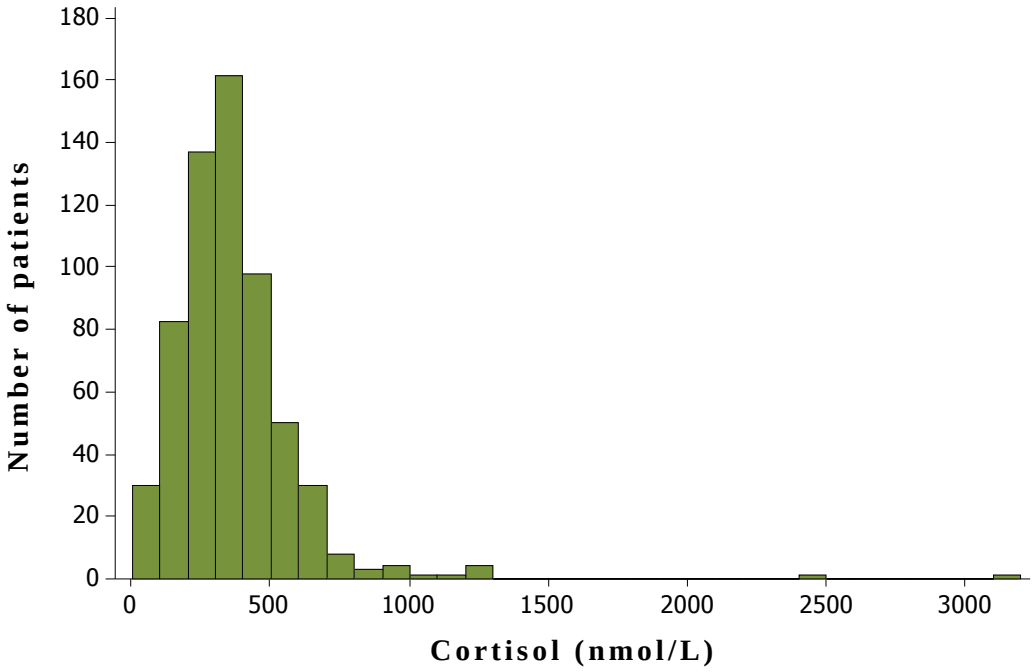


Figure 4-12. Frequency distribution histogram of cortisol levels in patients during hospital admission

4.3.3.3 11-deoxycortisol to cortisol ratio

An 11-deoxycortisol to cortisol ratio could be calculated for 600 of the 722 patients during hospital admission. The median (IQR) 11-deoxycortisol to cortisol ratio was 1.65 (0.96-2.88) $x10^{-3}$ (Figure 4-13) and the mean (SD) 11-deoxycortisol to cortisol ratio was 2.4 (2.8) $x10^{-3}$. A frequency distribution histogram of 11-deoxycortisol to cortisol ratio in these patients during the hospital admission is displayed in Figure 4-14. The minimum 11-deoxycortisol to cortisol ratio was 0.009 $x10^{-3}$ and the maximum 11-deoxycortisol to cortisol ratio was 34.8 $x10^{-3}$.

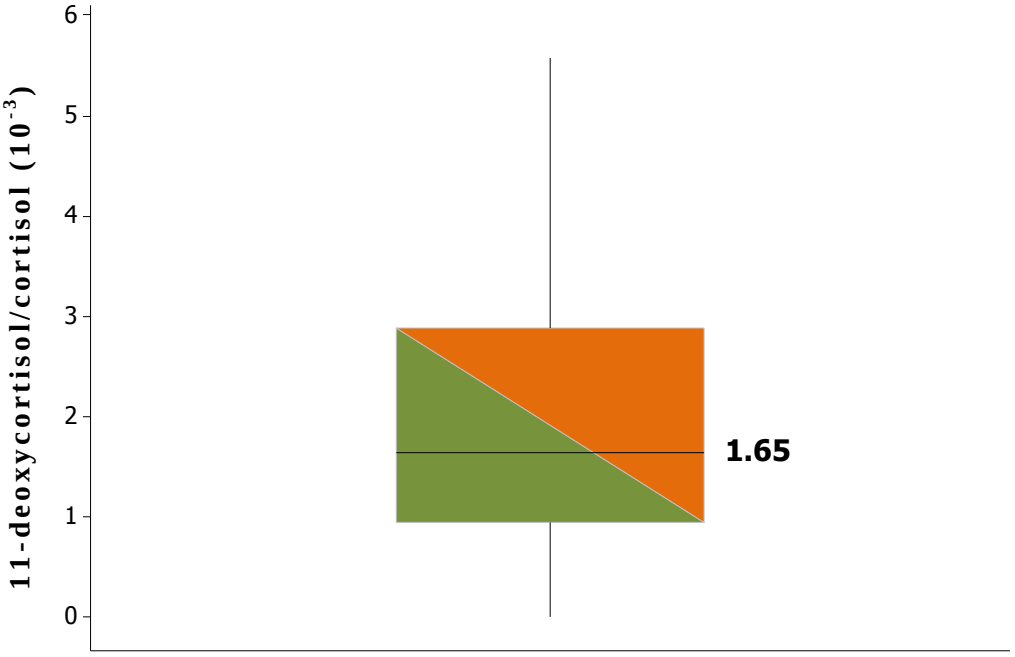


Figure 4-13. Box and whisker plot of 11-deoxycortisol to cortisol showing the 2.5, 25, 50, 75 and 97.5 centiles

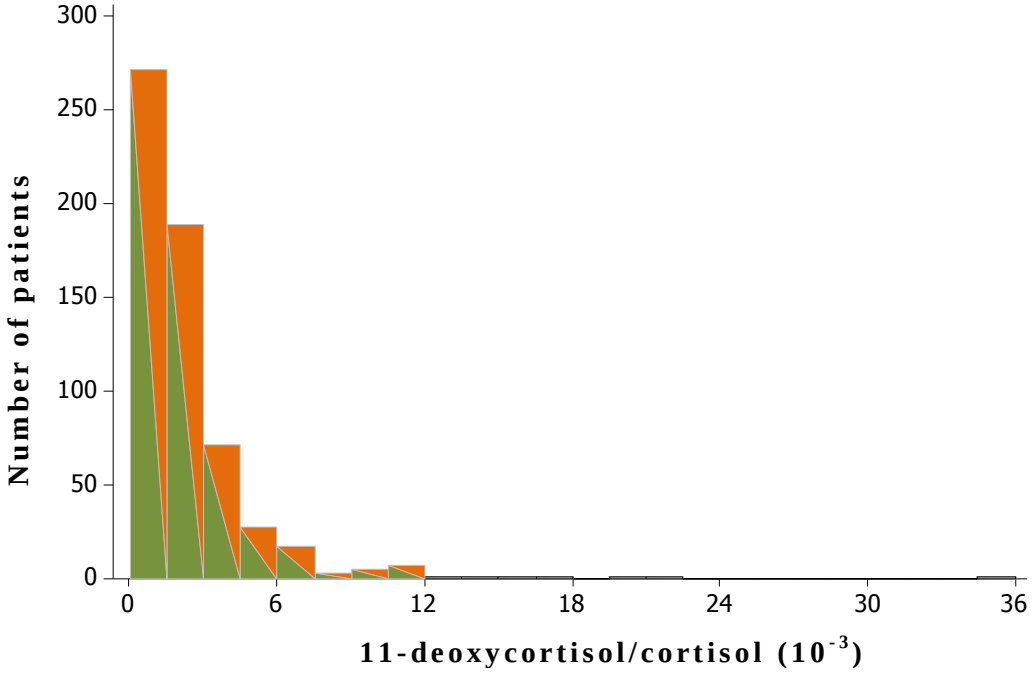


Figure 4-14. Frequency distribution histogram of 11-deoxycortisol to cortisol ratio in patients during hospital admission

4.3.3.4 Baseline levels of glucocorticoids according to background therapy with an oral glucocorticoid

Of the 722 patients studied during hospital admission, 26 were taking an oral glucocorticoid and 696 were not taking an oral glucocorticoid prior to admission. Cortisol levels were significantly lower in the former compared with the latter group (Table 4-3). The 11-deoxycortisol levels were not different between the two groups and the 11-deoxycortisol to cortisol ratio was lower in patients not taking oral glucocorticoid treatment compared with patients taking oral glucocorticoid therapy, but that failed to reach statistical significance.

Table 4-3. Levels of plasma glucocorticoids during hospital admission according to background therapy with an oral glucocorticoid

	All patients (n=722)	Oral glucocorticoid - Yes (n=26)	Oral glucocorticoid – No (n=696)	p-value†
11-deoxycortisol (pmol/L)	492.6 (275.5 – 929.6)	489.6 (97 – 1016)	496.8 (276.7 – 928.8)	0.296
Cortisol (nmol/L)	322.6 (226.0 – 444.4)	217.2 (108.5 – 336.2)	324.3 (233.3 – 451.3)	**0.001**
11-deoxycortisol/cortisol (10^{-3})	1.65 (0.96 - 2.88)	1.86 (1.07 – 3.36)	1.64 (0.96 – 2.88)	0.469

Values are presented as median (IQR)

† Mann-Whitney test was used for inter-group comparisons

4.3.4 Patient characteristics according to the levels of RAAS mediators and glucocorticoids during the hospital admission

The characteristics of the overall hospitalised cohort according to the levels of aldosterone and PRC and the aldosterone to PRC ratio during hospital admission are presented in the Appendix (Table 13-1 to Table 13-3). Similarly, the patient characteristics according to the levels of 11-deoxycortisol and cortisol and the 11-deoxycortisol to cortisol ratio are presented in the Appendix (Table 13-4 to Table 13-6)

4.4 Discussion

4.4.1 Baseline patient characteristics during hospital admission

The patients enrolled in this study were elderly and predominantly male. The average age (73 years) was similar to that reported in a large registry of patients with decompensated HF (333). Approximately half of the patients had a diagnosis of pre-existing HF prior to admission and a similar proportion of patients found to have history of previous MI. The latter is in accordance with previous findings, showing the predominance of ischaemic heart disease in patients with HF (334). The proportion of patients (approaching two thirds) with a history of hypertension, as well as the finding that the majority of patients on admission were normotensive or hypertensive is also consistent with previous findings (333).

Three quarters of patients were in NYHA class III or IV; however, considerably more patients were in NYHA class III than in class IV, reflecting a cohort of patients with predominantly moderate HF during hospital admission. Most patients had qualitative echocardiographic assessment of LV systolic function during hospital admission, which showed LVSD in approximately two thirds of the study population. That is slightly different from previous findings in hospitalised patients, showing that approximately half of patients with decompensated HF have LVSD (11). Quantitative assessment of LV systolic function was not performed in the current study and possible classification of patients with LVEF between 40% and 50% as having HFrSF may account for the higher proportion of patients with LVSD compared with previous reports. The proportion of patients (41%), found to be in AF (on a 12-lead ECG) during admission is similar to the EuroHeart Failure survey II reporting approximately thirty-nine percent of hospitalised patients for HF to be in AF prior to hospital admission (335). AF may cause or exacerbate the decompensation in HF patients by impairing the ventricular filling, either by loss of atrial contraction or by reducing the time of diastole when associated with rapid ventricular response.

A significant number of patients had abnormalities in their haematological and biochemical profile during hospital admission. More than half of the patients were anaemic according to World Health Organisation (WHO) criteria and had eGFR <60 ml/min/1.73m^2 indicating that anaemia and mild renal dysfunction are comorbid characteristics of patients hospitalised with HF. However, no data were available in this study about the number of patients with acute or chronic renal failure. That is of importance as a degree of acute kidney injury due to cardiac decompensation in patients with prior normal kidney function is expected to improve or resolve following treatment for HF in a number of patients. Moreover, patients studied during hospital admission were elderly and someone would expect a decline in eGFR in participants of that age group irrespective of HF. In the current study, patients hospitalised for other reasons were not examined and any comparisons of renal function between a control group and my group of patients hospitalised with HF were not available.

BNP levels on average were markedly elevated, reflecting high myocardial wall stress in these patients. In contrast, sodium levels were at the lower limit of normal, partially due to the water retention secondary to the activation of pathways with antidiuretic effects in patients with decompensated HF. In addition, more than half of patients had elevated troponin reflecting the degree of myocardial necrotic process. Myocardial stretch and neurohumoral activation have been suggested to contribute to myocardial injury and troponin release in patients with HF (336). Moreover, oxidative stress and inflammation have been linked with myocardial injury. In accordance with previous findings (337) (338), CRP levels were elevated during admission indicating that patients with decompensated HF manifest a systematic inflammatory response characterised by up-regulation in cytokine production.

Almost all patients in this cohort received diuretic therapy on admission to hospital. Interestingly, the proportion of patients taking an oral diuretic prior to hospital admission was higher than the proportion of patients with history of pre-existing HF. That may be due to empirical treatment of hypertension (previous history of hypertension was more prevalent than that of HF) or systemic congestion in patients with unconfirmed diagnosis of HF with a diuretic. More than half of the patients were taking a RAAS inhibitor (ACE inhibitor/ARB or aldosterone blocker) and approximately half of the patients were receiving a beta-blocker prior to admission. Finally, only a tiny minority were taking an oral glucocorticoid (2.2%).

4.4.2 Levels of RAAS mediators during hospital admission

Aldosterone levels were at the lower and PRC values at the upper limit of normal in my patients during hospital admission. Little is known about RAAS activation during worsening of HF and, especially, about activity of each of the components of this system during acute deterioration. What little prior information that has been published was obtained from patients treated with a RAAS blocker, confounding interpretation of the results (55) (339) (340). In my study, more than half of the patients were receiving an ACE inhibitor/ARB or aldosterone blocker prior to hospital admission. Treatment with a RAAS inhibitor has significant effects on the levels of RAAS mediators. In the current cohort, aldosterone levels were higher in patients treated with an aldosterone blocker but not with an ACE inhibitor or ARB and lower in patients treated with an ACE inhibitor or ARB but not with an aldosterone blocker. By contrast, PRC was higher in patients treated with an ACE inhibitor/ARB or aldosterone blocker and lower in patients taking neither an ACE inhibitor/ARB nor an aldosterone blocker. Aldosterone to PRC ratio was higher in patients not treated with a RAAS inhibitor and lower in patients taking a RAAS inhibitor i.e. the ratio was primarily determined by PRC but not by aldosterone.

The above findings are likely to be due to the discordant effects of the RAAS inhibiting agents on the levels of the RAAS components. ACE inhibitors and ARBs reduce aldosterone production by decreasing or antagonising angiotensin II; moreover, they increase PRC due to the absence of negative feedback of angiotensin II on renin production (34) (341). Aldosterone antagonists block the MR and increase aldosterone and PRC (288). Thus, in the presence of RAAS inhibitors the relationship between renin, which represents the main surrogate for the RAAS activity, and angiotensin II and aldosterone, which represent the main effectors of RAAS activity, becomes distorted. Clearly, even in the presence of RAAS inhibitors, higher levels of RAAS effectors do not lead to greater RAAS activity (i.e. greater receptor stimulation). Because of these observations, RAAS activity was further examined in relation to markers of HF severity in patients not taking a RAAS blocker prior to hospital admission and at the follow-up visit in chapter 6 & 7 respectively.

The review of recorded data about past medical history in extreme outliers with aldosterone levels above the upper limit of normal and extreme outliers with higher aldosterone to PRC ratio values revealed a patient with Conn's syndrome. This patient had the highest aldosterone to PRC ratio and elevated aldosterone levels both during hospital admission and the follow- up visit (see also section 5.3.2.1 & 5.3.2.3). The aldosterone to renin ratio is the most reliable screening tool for primary aldosteronism as a secondary cause of hypertension (342). Recently, the feasibility of using PRC instead of PRA for the calculation of aldosterone to renin ratio has been shown as a first-line screen in patients for primary aldosteronism (318). In the current study, PRC was at the lowest detectable level and aldosterone was above the normal range despite treatment with an ACE inhibitor and aldosterone blocker in the HF patient with history of Conn's syndrome. Autonomous aldosterone secretion due to adrenal adenoma is likely to explain the elevated aldosterone levels with secondary volume expansion-induced PRC suppression despite the use of RAAS

inhibition in that participant. Review of medical notes confirmed the presence of adrenal adenoma in that patient during enrolment in the study. History of Conn's syndrome or primary aldosteronism was not present in the other extreme outliers with higher aldosterone to PRC ratio or with aldosterone levels above normal. In patients of the latter group, PRC values were elevated (apart from the patient with Conn's syndrome) reflecting that the up-stream RAAS components led to elevated aldosterone levels. On the other hand, in the group of extreme outliers with higher aldosterone to PRC ratio (apart from the patient with Conn's syndrome), PRC was suppressed (5mIU/L) and aldosterone levels were high but within normal range. The majority of these patients had history of hypertension and some of them might have low renin hypertension due to primary aldosteronism, although that was not confirmed in the medical history. Finally, data entry errors cannot be totally excluded; hence, some of the extreme outliers might be due to that.

4.4.3 Levels of glucocorticoids during hospital admission

Levels of plasma cortisol levels were within the normal range during hospital admission. This was initially surprising and seemed to be in contrast to two prior studies of untreated patients with severe congestive HF (69) (70). However, these studies were small and additionally included patients with acute HF secondary to MI. Nevertheless, due to the fact that blood samples were collected 24 to 48 hours after hospital admission in the current study, the stimulation of the HPA axis may have subsided. Indeed, in patients with cardiogenic pulmonary oedema, cortisol was markedly raised an hour after the onset of symptoms and gradually returned within the normal range in 12 hours following hospital admission (343). Similarly, a decline in cortisol levels following 48 hours after admission has been reported in patients with an uncomplicated MI (344). These findings suggest that normal cortisol levels found in my study might be due to the time of blood sampling, as all patients had blood samples taken 24 to 48 hours following the initiation of in-hospital treatment. Interestingly,

cortisol levels in my patients are similar to the levels previously reported in patients with *chronic* HF (72) (73). Indeed, perhaps surprisingly, they are similar to ambulatory cortisol levels measured in morning hours in healthy volunteers (section 3.3.1). Cortisol is a non-specific indicator of stress and the findings of this study indicate that no major activation of HPA axis in patients with decompensated HF was present within 48 hours after admission.

Glucocorticoid levels during hospital admission will be further examined in relation to patient characteristics and features of HF severity in chapter 8. Finally, the prognostic importance of glucocorticoids in patients with decompensated HF will be studied in chapter 10.

5. Chapter Five - Patient characteristics at the follow-up visit

5.1 Introduction

The main aim of this chapter is to present the clinical characteristics of patients with HF at the follow-up visit. In this chapter, I also show the plasma levels of RAAS components and glucocorticoids in these patients. In addition, I present these levels according to background therapy with a RAAS inhibitor and oral glucocorticoid therapy respectively. Lastly, I compare the levels of glucocorticoids in the subgroup of patients who had blood samples collected in the morning both during hospital admission and at follow-up.

5.2 Methods

5.2.1 Study design and laboratory measurements

Details of the study participants and the laboratory measurements were presented previously in sections 2.4.1 & 2.4.2.

5.2.1 Statistical analysis

All patient characteristics are expressed as median (IQR) for continuous and absolute number (percentage) for categorical variables. The inter-group comparisons were carried out by the Mann-Whitney and Kruskal-Wallis test for continuous variables as appropriate and by the χ^2 test for categorical variables. For the comparisons of glucocorticoid levels and patient characteristics between hospital admission and the follow-up visit in the group of patients who had blood taken in the morning at both stages, Wilcoxon matched pairs test and McNemar test were employed for continuous and categorical variables respectively. A p-value <0.05 was considered significant for all analyses. Statistical analyses were performed with Minitab version 15.

5.3 Results

5.3.1 Baseline patient characteristics during follow-up

Of the 722 patients enrolled in the study during hospital admission, 269 patients (37.3%) failed to attend the follow-up visit (Figure 4.1). Almost a fifth of patients included in the study (n=136) refused to return to the BHF Glasgow Cardiovascular Research Centre for the follow-up visit. Just above a tenth of the enrolled patients (n=74) died prior to the study visit appointment. Lastly, approximately eight percent (n=59) of patients included in the study were not in position to attend the follow-up visit due to deterioration in their health status.

The characteristics of the 453 patients who attended the follow-up visit are presented in Table 5-1. The median age (IQR) was 72 (66-78) years and forty percent were women. Approximately two thirds of patients (63.6%) were in NYHA class II and one third (32.9%) were in NYHA class III. These findings were in contrast to those of hospital admission, where the majority of patients were in NYHA class III and IV. Similar to the hospitalised cohort, over forty percent of patients had a history of MI (43%), two-thirds had pre-existing hypertension and almost a third of patients had history of diabetes after discharge. While over half of the patients (53%) at follow-up had a history of AF, only a third (34.4%) had this arrhythmia on their screening ECG.

The median (IQR) BMI was 27.6 (23.8-32.6) kg/m^2. Approximately two thirds of patients were overweight (BMI 25-30 kg/m^2) or obese (BMI >30 kg/m^2) and a small proportion (< 3%) of patients were underweight (BMI <18.5 kg/m^2).

The median (IQR) SBP was 129 (114-144) mmHg. Approximately a third (32.9%) of patients had SBP ≥ 140mmHg, proportion that was smaller compared with the proportion of patients (41%) with SBP ≥ 140mmHg during hospital admission. A similar proportion (2%) to

hospitalised cohort had SBP <90mmHg after discharge. In contrast, a smaller percentage of patients (5%) at follow-up had DBP >90mmHg com**p**ared with hospital admission (20%). The median (IQR) pulse rate was 74 (65-86) bpm. Less than a tenth of patients (6.1%) were tachycardic (pulse rate >100 bpm), whilst a greater proportion (16.1%) of patients were bradycardic (pulse rate <60bpm). The above findings were markedly different from hospital admission, where the median pulse rate (86bpm) was higher and a third of patients were tachycardic.

The median (IQR) LVEF of the post-discharge cohort was 40 (38 - 41) % and the mean (SD) LVEF was 39.7 (11.8) %. Almost eighty percent (78.9%) of patients had LVEF < 50% and approximately a third (32.7%) of patients had LVEF <35%.

The median BNP (IQR) during follow-up was 396 (206 - 813) pg/ml. That was markedly decreased compared with the hospital admission, where the median (IQR) BNP was 871pg/ml (391 - 1819). Sodium levels were well within the normal range after discharge. Approximately a tenth of patients were hyponatraemic and less than one percent were hypernatraemic in contrast with the hospitalised cohort, where approximately a fifth of patients were hyponatraemic and four percent were hypernatraemic. The median eGFR (IQR) was 59 ml/min/1.73m^2 with half of the patients having eGFR <60 ml/min/1.73m^2 and less than a tenth having an eGFR <30 ml/min/1.73m^2. Similar to the hospitalised cohort, although the median creatinine (106 µmol/L) was within the normal range, the median urea (8.6 mmol/L) was raised.

A considerably smaller proportion of patients (18%) after discharge had elevated troponin compared with hospital admission. The median (IQR) CRP at the study visit was 5.2 (9.4) mg/L and the median of TSH and lipids were within the normal ranges.

The median (IQR) haemoglobin was 12.5 (11.2-13.6) g/dl. Just over half of the male participants (51%) and almost half of the female participants (46%) had anaemia, defined as haemoglobin <13g/dl and haemoglobin <12g/dl respectively.

Almost all patients of the post-discharge cohort (98.2%) were taking an oral diuretic. More than seventy percent (72.8%) of patients were taking an ACE inhibitor and eighty percent were taking an ACE inhibitor or ARB during follow-up. More than two thirds of patients (68.2%) were taking a beta-blocker, while only fourteen percent were taking an aldosterone blocker. A minority of patients (3.1%) were treated with a steroid tablet after discharge.

Table 5-1. Patient (n=453) characteristics at the follow-up visit

Variable	Median or number of patients	IQR or %
Age (years)	72	66 - 78
Female gender	181	40
NYHA class		
I	12	2.6
II	288	63.6
III	149	32.9
IV	4	0.9
Medical history		
HF	188	41.5
MI	195	43
Angina	248	54.7
Diabetes mellitus	227	50.1
Hypertension	296	65.3
AF	240	53
CVA/TIA	91	20.1
Physiological measurements		
BMI (kg/m^2)	27.6	23.8 - 32.6
Weight (kg)	75	62 - 89
Pulse rate (bpm)	74	65 - 86
SBP (mmHg)	129	114 - 144
DBP (mmHg)	67	58 - 76
ECG rhythm		
SR	269	59.4
AF	165	34.4
Echo measurements		
LVEF (%)	40	31 - 48
Laboratory measurements (blood)		
BNP (pg/ml)	396	206 - 813
Troponin I ≥ 0.04 (µg/L)	82	18.1
Sodium (mmol/L)	139	137 - 141
Potassium (mmol/L)	4.3	3.8 - 4.3
Urea (mmol/L)	8.6	6.5 - 11.9
Creatinine (µmol/L)	106	87 - 130.5
eGFR ($ml/min/1.73m^2$)	59	43 - 60
eGFR <60$ml/min/1.73m^2$	230	50.8

Variable	Median or number of patients	IQR or %
Cholesterol (total) (mmol/L)	4.0	3.3 - 4.9
HDL (mmol/L)	1.1	0.8 - 1.3
CRP (mg/L)	5.2	2.6 - 12
TSH (mIU/L)	1.5	0.9 - 2.4
Haemoglobin (g/dl)	12.5	11.2 - 13.6
Cardiovascular Medication		
Diuretic	445	98.2
Furosemide	412	90.9
ACE inhibitor	330	72.8
ACE inhibitor or ARB	363	80.1
Aldosterone blocker	64	14.1
Beta-blocker	309	68.2
Digoxin	115	25.4
Anti-arrhythmic	26	5.7
Aspirin	253	55.8
Statin	335	74
Non-cardiovascular medication		
Steroid tablets	14	3.1

Continuous variables are presented as median (IQR). Categorical variables are presented as number percentage).

Abbreviations: NYHA, New York Heart Association; HF, Heart Failure; MI, myocardial infarction; AF, atrial fibrillation; CVA, cerebrovascular accident; TIA, transient ischaemic attack; BMI, body mass index; bpm, beats per minute; SBP, systolic blood pressure; DBP, diastolic blood pressure; SR, sinus rhythm; LVEF, left ventricular ejection fraction; BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; CRP, C-reactive protein; TSH, thyroid stimulating hormone; ACE, angiotensin-converting enzyme ARB, angiotensin receptor blocker

5.3.2 RAAS activation during follow-up

Levels of plasma aldosterone and PRC and the aldosterone to PRC ratio at follow-up are presented below.

5.3.2.1 Aldosterone

An aldosterone level measured at the follow-up visit was available in 428 of 453 patients. The median (IQR) aldosterone concentration was 143 (76.7 - 267) pmol/L (Figure 5-1) and the mean (SD) aldosterone concentration was 215.8 (298.5) pmol/L. A frequency distribution histogram of aldosterone levels in these patients is displayed in Figure 5-2. The distribution of aldosterone concentrations was positively skewed. The minimum aldosterone value was 2.5 pmol/L and the maximum aldosterone value was 3894.5 pmol/L. Almost all patients (98.5%) had aldosterone levels within the normal range and only six patients (1.5%) had aldosterone levels above the upper limit of normal (937 pmol/L). Review of information about the past medical history in the latter group revealed the presence of Conn's syndrome in one of these patients (aldosterone level of 2056.4 pmol/L). That was the same participant who was previously identified to have Conn's syndrome during hospital admission (see section 4.3.2.1 and 4.3.2.3). PRC was low (5 mIU/L) and aldosterone to PRC ratio was markedly elevated (411.3) in that patient at the follow-up visit similar to the hospital admission.

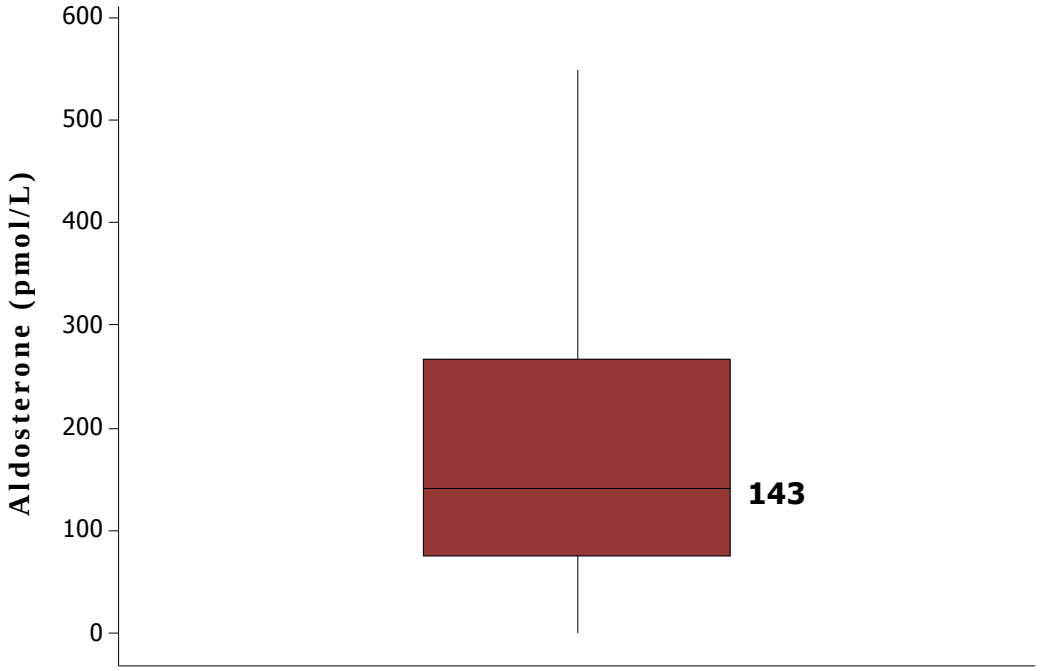


Figure 5-1. Box and whisker plot of aldosterone concentrations showing the 2.5, 25, 50, 75 and 97.5 centiles

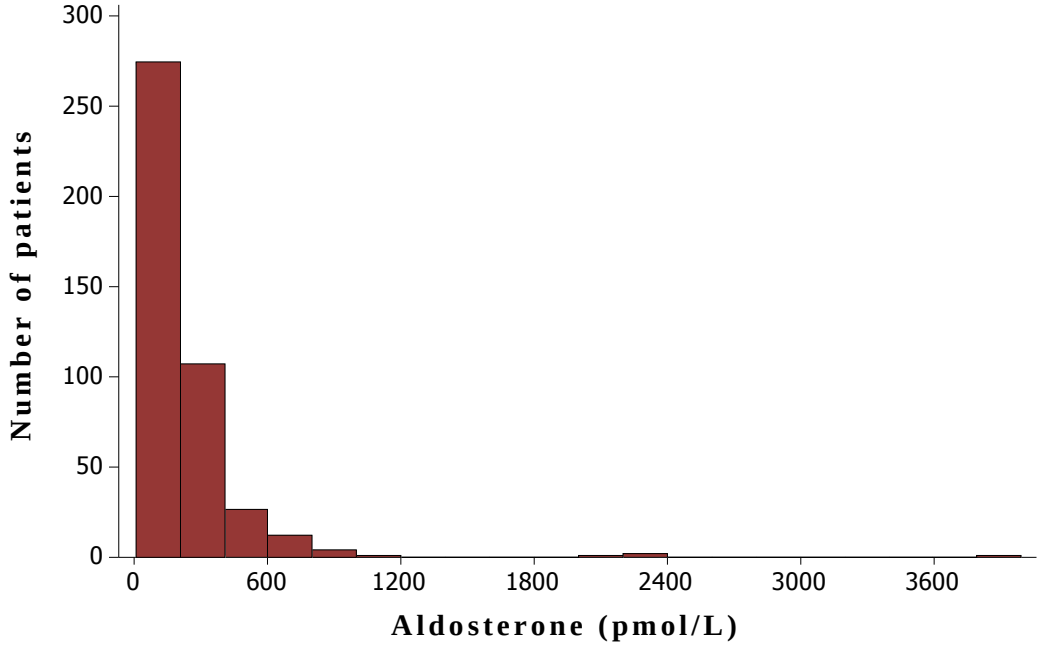


Figure 5-2. Frequency distribution histogram of aldosterone concentrations in the overall cohort

5.3.2.2 PRC

PRC measured at the follow-up visit was available in 445 of 453 patients. The median (IQR) PRC was 92.8 (26.1 - 327.8) mIU/L (Figure 5-3) and the mean (SD) PRC was 490 (1147.8) mIU/L. A frequency distribution histogram of PRC in these patients is displayed in Figure 5-4. The distribution of PRC values was positively skewed. The minimum PRC was 5.0 mIU/L and the maximum PRC was 8326 mIU/L. Approximately a third (33%) of patients had a PRC within the normal range and two-thirds had a PRC above the upper limit of normal (44.9 mIU/L).

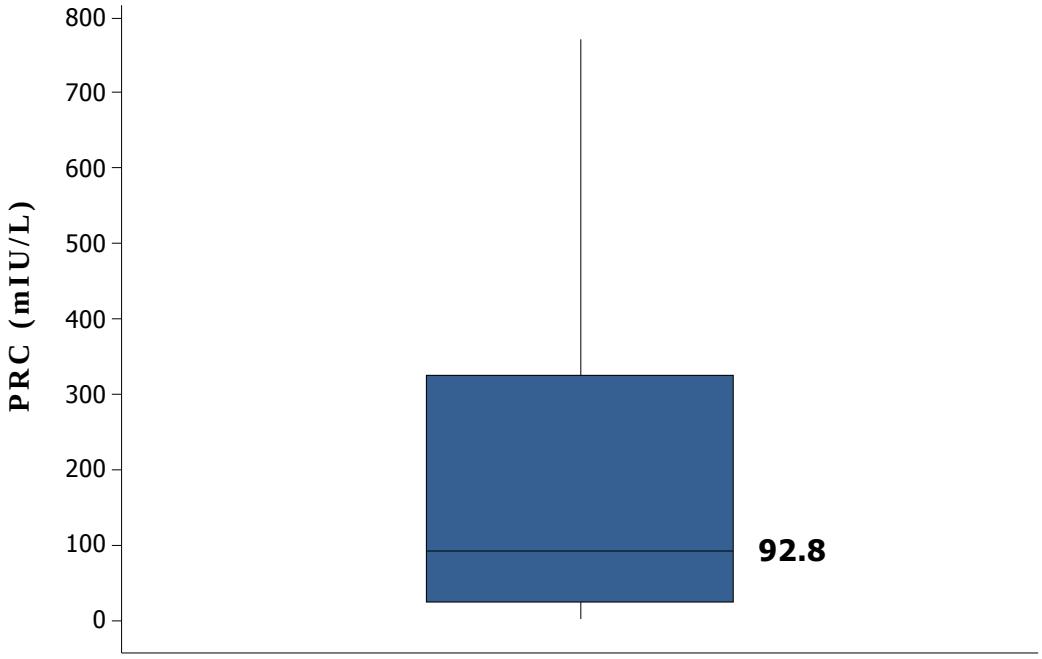


Figure 5-3. Box and whisker plot of PRC showing the 2.5, 25, 50, 75 and 97.5 centiles

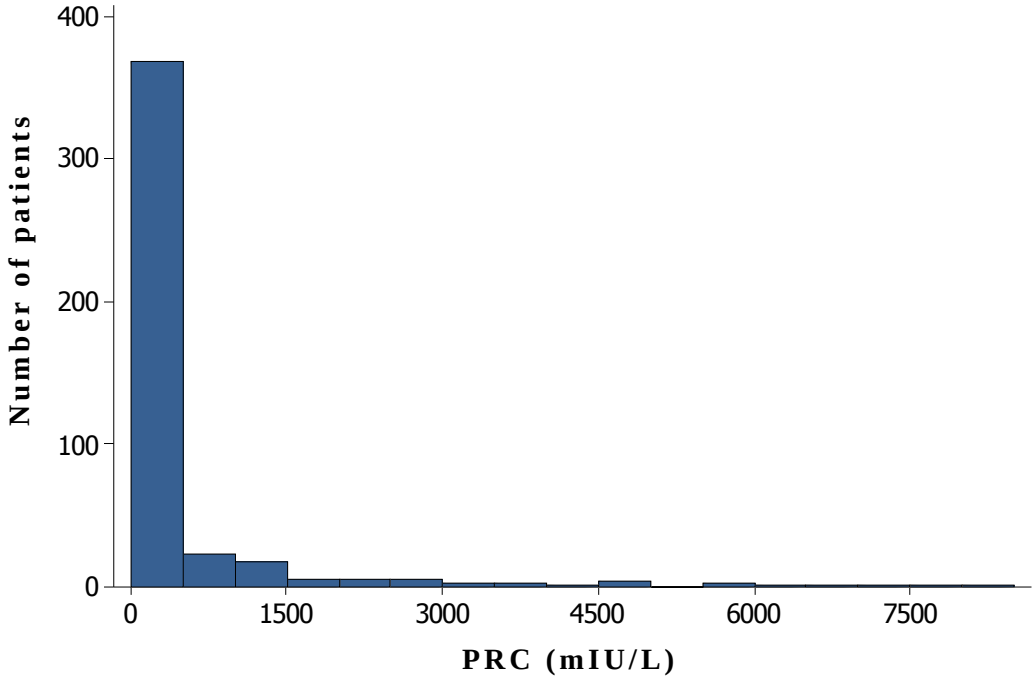


Figure 5-4. Frequency distribution histogram of PRC in the overall cohort

5.3.2.3 Aldosterone to PRC ratio

An aldosterone to PRC ratio could be calculated for 426 of the 453 patients. The median (IQR) aldosterone to PRC value was 1.51 (0.4 - 5.3) (Figure 5-5) and the mean (SD) aldosterone to PRC value was 6.8 (23.1). A frequency distribution histogram of aldosterone to PRC values in these patients is displayed in Figure 5-6. The distribution of aldosterone to PRC values was positively skewed. The minimum aldosterone to PRC ratio was 0.004 and the maximum aldosterone to PRC ratio was 411.3. The patient with the maximum aldosterone to PRC ratio was the participant with a history of Conn's syndrome (see section 5.3.2.1, 4.3.2.3 and 4.3.2.1).

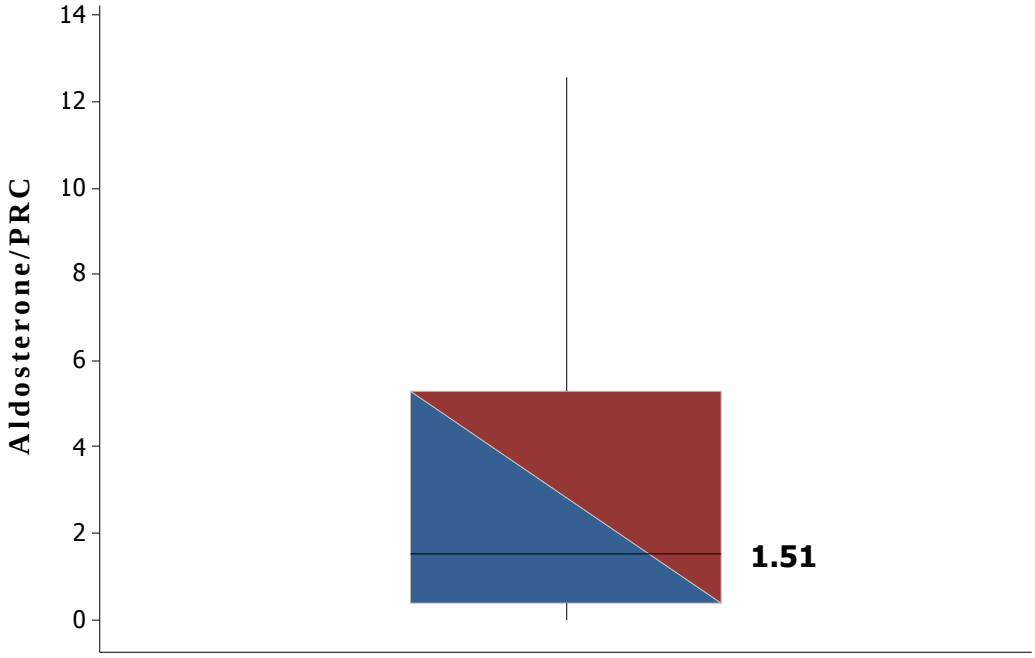


Figure 5-5. Box and whisker plot of aldosterone to PRC ratio showing the 2.5, 25, 50, 75 and 97.5 centiles

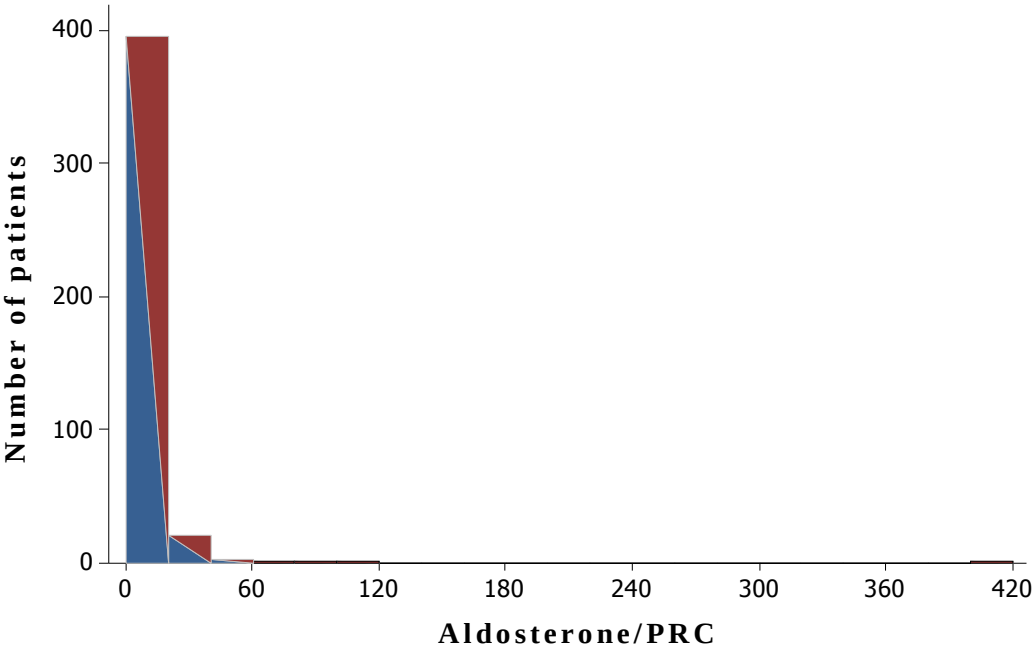


Figure 5-6. Frequency distribution histogram of aldosterone to PRC ratio in the overall cohort

5.3.2.4 RAAS activation according to background therapy with a RAAS inhibitor

Levels of RAAS mediators according to background therapy with a RAAS inhibitor are presented in Table 5-2. Similar to the hospitalised cohort, aldosterone levels were higher in patients taking an aldosterone blocker but not an ACE inhibitor or ARB and lower in patients taking an ACE inhibitor or ARB but not an aldosterone blocker prior to admission. PRC was higher in patients receiving an ACE inhibitor/ARB or aldosterone blocker and lower in patients receiving neither an ACE inhibitor/ARB nor an aldosterone blocker. Conversely, aldosterone to PRC ratio was lower in patients treated with a RAAS inhibitor and higher in patients not taking a RAAS inhibitor prior to hospital admission.

Table 5-2. Levels of RAAS mediators at follow-up by background therapy with an ACE inhibitor/ARB or aldosterone blocker

Variable	All patients (n=453)	ACE inhibitor/ARB (No) Aldosterone blocker (No) (n=79)	ACE inhibitor/ARB (Yes) Aldosterone blocker (No) (n=310)	ACE inhibitor/ARB (No) Aldosterone blocker (Yes) (n=11)	ACE inhibitor/ARB (Yes) Aldosterone blocker (Yes) (n=53)	p-value†
Aldosterone (pmol/L)	143 (76.7 - 267)	182.4 (92.6 - 329)	120.4 (68.8 - 207.5)	405.5 (247.4 - 755.5)	252.4 (130.2 - 450.4)	**<0.001**
PRC (mIU/L)	92.8 (26.1 - 327.8)	47.5 (18.9 - 107.1)	87.6 (25.6 - 346.6)	256.7 (113 - 482.7)	320 (101 - 1460)	**<0.001**
Aldosterone/ PRC	1.51 (0.4 - 5.3)	3.74 (1.88 - 9.73)	1.25 (0.29 - 4.96)	2.43 (0.28 - 6.46)	0.66 (0.2 - 2.47)	**<0.001**

Values are presented as median (IQR)
† Kruskal-Wallis test was used for inter-group comparisons

5.3.3 Levels of glucocorticoid during follow-up

Levels of plasma 11-deoxycortisol and cortisol, and the 11-deoxycortisol to cortisol ratio at follow-up are presented below.

5.3.3.1 11-deoxycortisol

An 11-deoxycortisol level measured at the follow-up visit was available in 417 of 453 patients. The median (IQR) 11-deoxycortisol concentration was 465.7 (291 - 730) pmol/L (Figure 5-7) and the mean (SD) 11-deoxycortisol concentration was 657.5 (935.3) pmol/L. A frequency distribution histogram of 11-deoxycortisol levels in these patients is presented in Figure 5-8. The minimum 11-deoxycortisol value was 10.8 pmol/L and the maximum 11-deoxycortisol value was 14885 pmol/L. The majority of patients (96%) had 11-deoxycortisol levels within the normal range (0 - 2017 pmol/L).

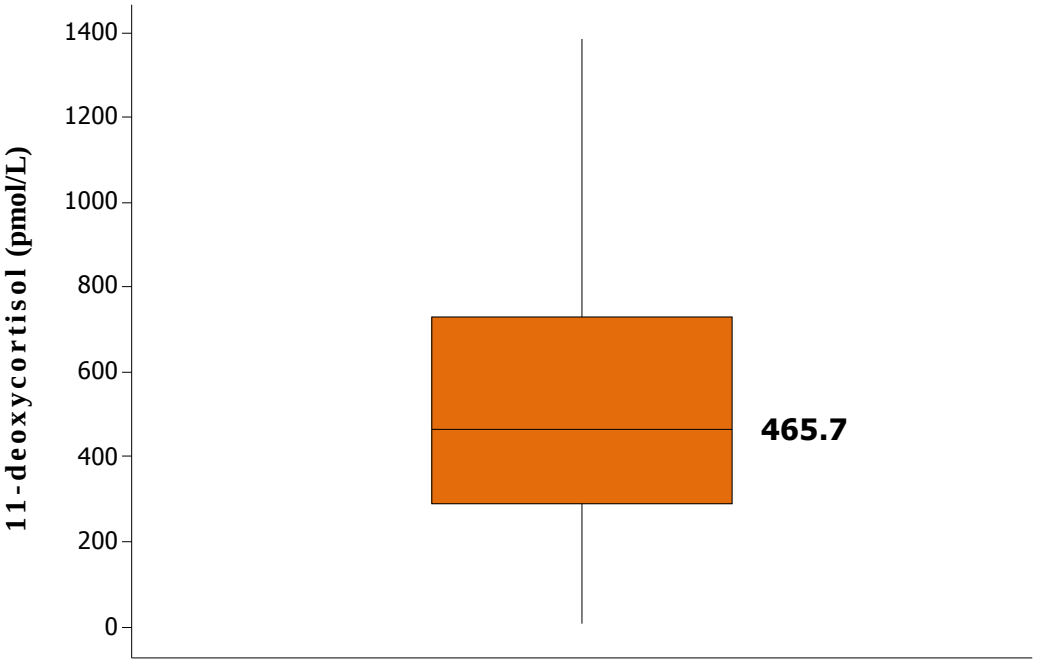


Figure 5-7. Box and whisker plot of 11-deoxycortisol showing the 2.5, 25, 50, 75 and 97.5 centiles

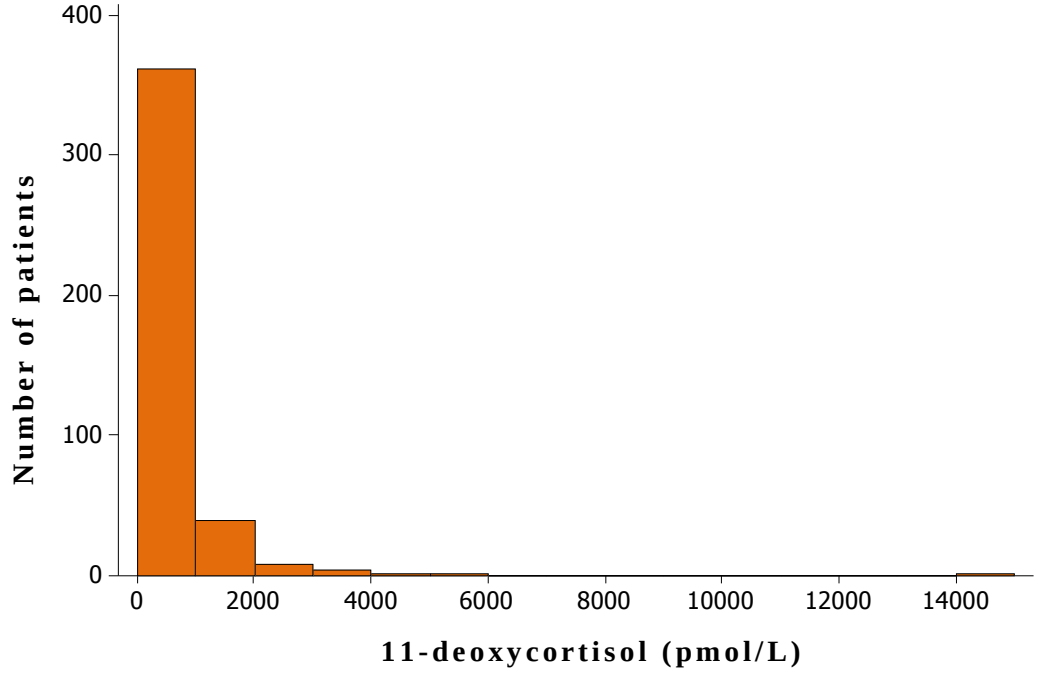


Figure 5-8. Frequency distribution histogram of 11-deoxycortisol concentrations in the overall cohort

5.3.3.2 Cortisol

A cortisol level measured at the follow-up visit was available in 427 of 453 patients. The median (IQR) cortisol concentration was 215.8 (149-295.6) nmol/L (Figure 5-9) and the mean (SD) aldosterone concentration was 236.6 (140.2) nmol/L. A frequency distribution histogram of cortisol levels in these patients is displayed in Figure 5-10. The minimum cortisol value was 3.3 nmol/L and the maximum cortisol value was 1166.4 nmol/L. Almost all patients (99.3%) had cortisol levels within the normal range (0 – 823nmol/L).

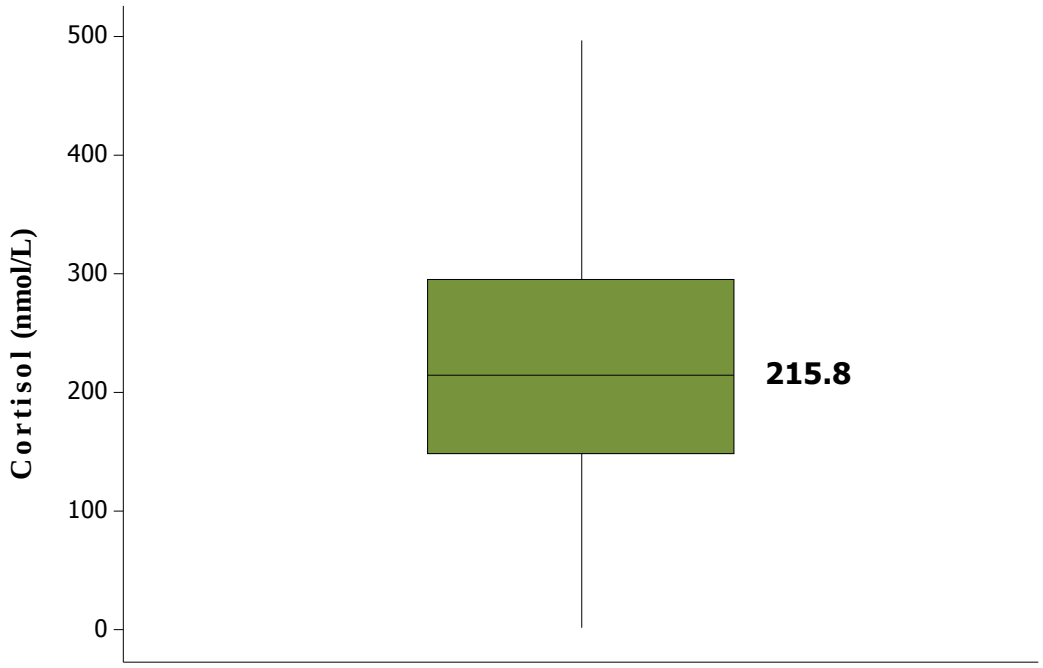


Figure 5-9. Box and whisker plot of cortisol concentrations showing the 2.5, 25, 50, 75 and 97.5 centiles

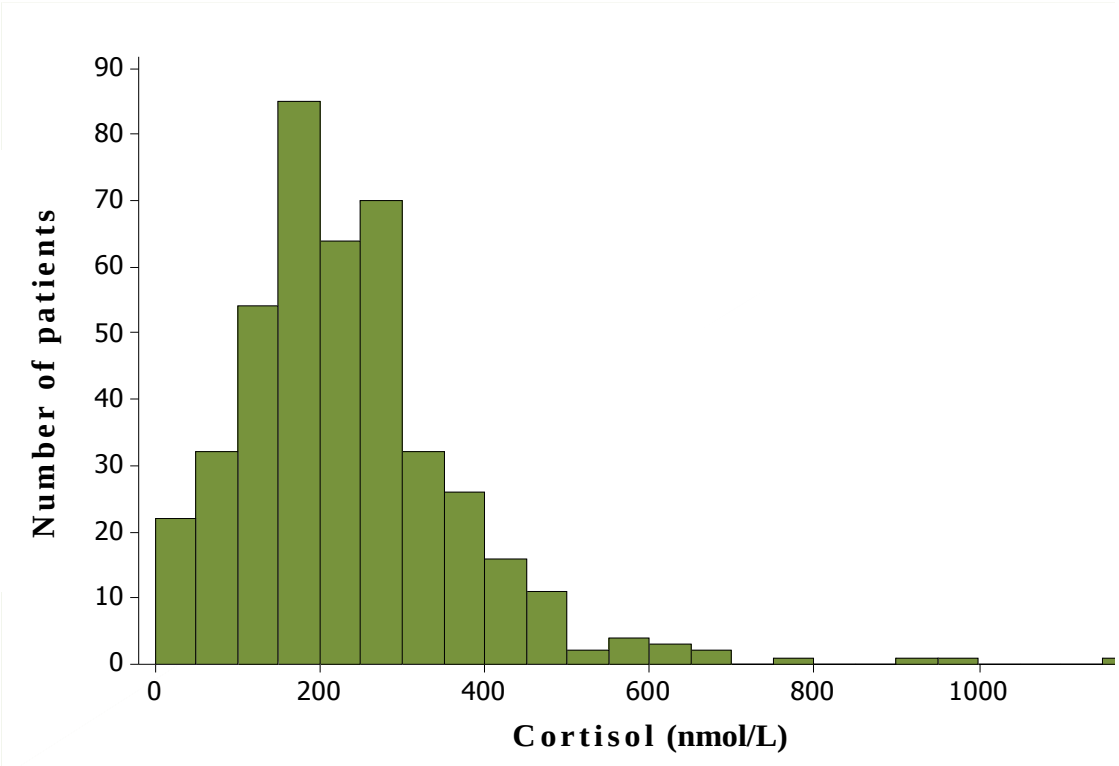


Figure 5-10. Frequency distribution histogram of cortisol concentrations in the overall cohort

5.3.3.3 11-deoxycortisol to cortisol ratio

An 11-deoxycortisol to cortisol ratio could be calculated for 415 of the 453 patients at the follow-up visit. The median (IQR) 11-deoxycortisol to cortisol was 2.33 (1.39 -3.76) $x10^{-3}$ (Figure 5-11) and the mean (SD) value was 3.71 (7.91) $x10^{-3}$. A frequency distribution histogram of 11-deoxycortisol to cortisol ratio in these patients is presented in Figure 5-12. The minimum 11-deoxycortisol to PRC ratio was 0.07 $x10^{-3}$ and the maximum 11-deoxycortisol to cortisol ratio was 118.4 $x10^{-3}$.

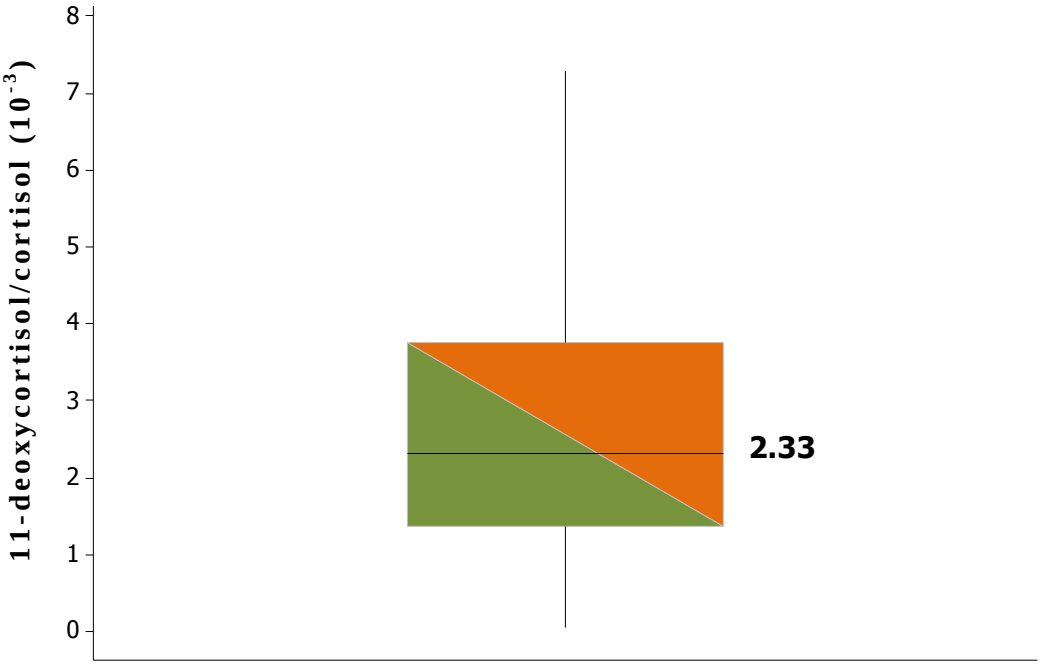


Figure 5-11. Box and whisker plot of 11-deoxycortisol to cortisol ratio showing the 2.5, 25, 50, 75 and 97.5 centiles

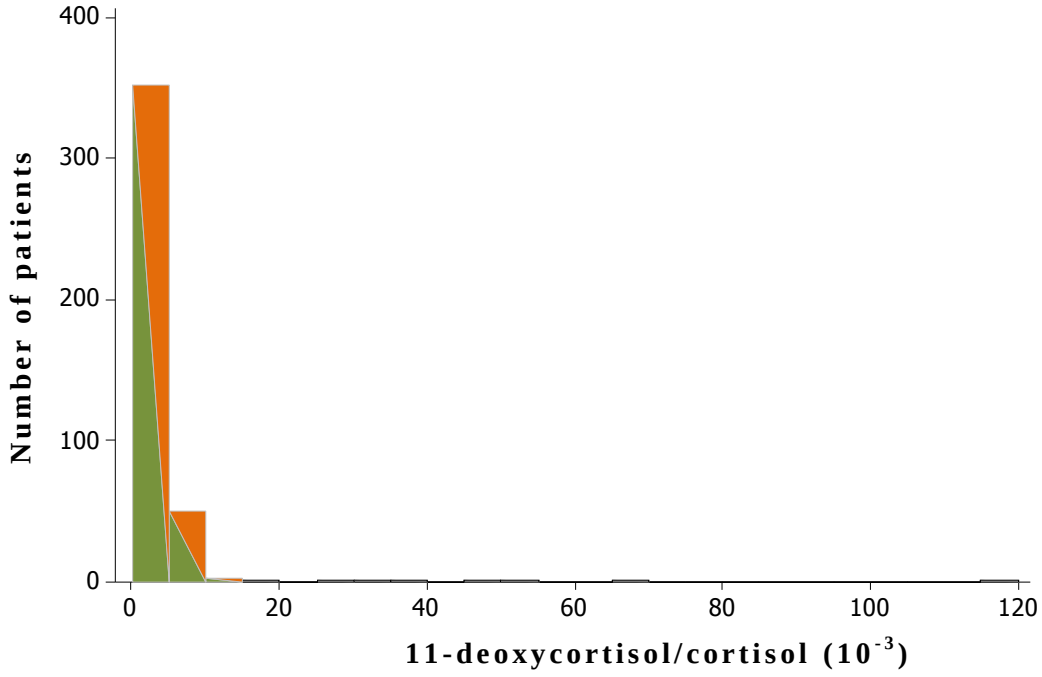


Figure 5-12. Frequency distribution histogram of 11-deoxycortisol to cortisol ratio in the overall cohort

5.3.3.4 Levels of glucocorticoids according to background therapy with an oral glucocorticoid

Of the 453 patients studied during the hospital admission, 14 were taking an oral glucocorticoid and 439 were not taking an oral glucocorticoid during follow-up (Table 5-3). Cortisol and 11-deoxycortisol levels were significantly lower in the former compared with the latter group. The 11-deoxycortisol to cortisol ratio was lower in patients not taking oral glucocorticoid treatment compared with patients taking oral glucocorticoid therapy, but that did not reach statistical significance.

Table 5-3. Levels of glucocorticoids in the overall cohort during follow-up according to background therapy with an oral glucocorticoid

Variable	All patients (n=453)	Oral glucocorticoid - Yes (n=14)	Oral glucocorticoid - No (n=439)	p-value†
11-deoxycortisol (pmol/L)	465.7 (291-730)	218.9 (88.8-489.3)	468.8 (296.8-742.0)	**0.004**
Cortisol (nmol/L)	215.8 (149-295.6)	71.8 (23.2-159.3)	218.5 (151.0-297.3)	**<0.001**
11-deoxycortisol/cortisol (10^{-3})	2.33 (1.39-3.76)	4.0 (1.3-14.9)	2.3 (1.4-3.7)	0.102

Values are presented as median (IQR)

† Mann-Whitney test was used for inter-group comparisons

5.3.4 Patient characteristics according to the levels of RAAS components and glucocorticoids during follow-up

The characteristics of the post-discharge cohort according to the levels of aldosterone and PRC and the aldosterone to PRC ratio at follow-up are presented in the Appendix (Table 13-7 to Table 13-9). Similarly, the patient characteristics according to the levels of 11-deoxycortisol and cortisol and the 11-deoxycortisol to cortisol ratio are presented in the Appendix (Table 13-10 to Table 13-12).

5.3.5 Levels of glucocorticoids at follow-up measured in the morning – comparison with glucocorticoid levels during the hospital admission

Of the 453 patients studied during follow-up, 31 had blood samples taken during morning hours and 422 had blood samples taken during afternoon hours (Table 5-4).

Cortisol levels were higher in the group of patients studied in the morning compared with patients studied in the afternoon at follow-up. Patients of the former group were less likely to have previous history of hypertension and were more likely to have lower HDL compared with patients of the latter group.

Table 5-4. Levels of glucocorticoids and patient characteristics during follow-up according to the time of blood sampling

Variable	Blood sampling in the morning (n=31)	Blood sampling in the afternoon (n=422)	Overall cohort – follow-up (n=453)	p-value†
Age (years)	71 (60 – 78)	72 (66 - 78)	72 (66 - 78)	0.619
Female gender	11 (35.5)	170 (40.3)	181 (40)	0.598
NYHA class				
I	1 (3)	11(2.6)	12 (2.6)	- ¥
II	19 (61)	269 (63.7)	288 (63.6)	0.784
III	11 (35.5)	138 (32.7)	149 (32.9)	0.750
IV	0 (0)	4 (1.0)	4 (0.9)	- ¥
Medical history				
HF	12 (38.7)	176 (41.7)	188 (41.5)	0.744
MI	18 (58.1)	177 (41.9)	195 (43)	0.082
Angina	21 (67.7)	227 (53.8)	248 (54.8)	0.132
Diabetes mellitus	11 (35.5)	132 (31.3)	143 (31.6)	0.627
Hypertension	14 (45.2)	282 (66.8)	296 (65.3)	**0.014**
AF	17 (54.8)	223 (52.8)	240 (53)	0.830
CVA/TIA	8 (25.8)	83 (19.7)	91 (20.1)	0.410
Physiological measurements				
BMI (kg/m^2)	25.6 (23.9 - 30.9)	27.7 (23.8 - 32.7)	27.6 (23.8 - 32.6)	0.287
Weight (kg)	70 (63 – 88)	75.1 (62 - 89)	75 (62 - 89)	0.492
Pulse rate (bpm)	74 (62 – 86)	74 (65 - 86.3)	74 (65 - 86)	0.654

Variable	Blood sampling in the morning (n=31)	Blood sampling in the afternoon (n=422)	Overall cohort – follow-up (n=453)	p-value†
SBP (mmHg)	125 (107 - 140)	129 (115 - 144.3)	129 (114 - 144)	0.167
DBP (mmHg)	68 (56 – 75)	67 (58 - 76)	67 (58 - 76)	0.691
ECG rhythm				
SR	16 (51.6)	253 (60)	269 (59.4)	0.361
AF	13 (41.9)	152 (36)	165 (36.4)	0.509
Echo measurements				
LVEF (%)	37 (29 – 44)	40 (31.8 - 48)	40 (31 - 48)	0.171
Laboratory measurements (blood)				
BNP (pg/ml)	423 (187 - 1015)	395.5 (207 - 811.5)	396 (206 - 813)	0.807
Sodium (mmol/L)	140 (138 - 141)	139 (137 - 141)	139 (137 - 141)	0.461
Potassium (mmol/L)	4.2 (3.8 - 4.4)	4.0 (3.8 - 4.3)	4.1 (3.8 - 4.3)	0.592
Urea (mmol/L)	8.6 (6.4 - 11.6)	8.7 (6.5 - 11.9)	8.6 (6.5 - 11.9)	0.894
Creatinine (µmol/L)	110 (87 - 137)	106 (87 - 130)	106 (97 - 130.5)	0.924
eGFR (ml/min/1.73m^2)	60 (44 – 60)	59 (43 - 60)	59 (43 - 60)	0.921
eGFR <60ml/min/1.73m^2	15 (48)	215 (51)	230 (50.8)	0.783
Cholesterol (total) (mmol/L)	3.8 (3.2 - 4.9)	4.1 (3.3 - 4.9)	4.0 (3.3 - 4.9)	0.414
HDL (mmol/L)	1.0 (0.8 - 1.1)	1.1 (0.9 - 1.4)	1.1 (0.8 - 1.3)	**0.047**
CRP (mg/L)	5.9 (1.8 – 13)	5.2 (2.7 - 12)	5.2 (2.6 - 12)	0.621
TSH (mIU/L)	1.6 (0.9 - 2.3)	1.5 (0.9 - 2.4)	1.5 (0.9 - 2.4)	0.674
Cortisol (nmol/L)	274.5 (170.4 - 374.4)	209.8 (147.7 - 292.3)	215.8 (149 - 295.6)	**0.010**

Variable	Blood sampling in the morning (n=31)	Blood sampling in the afternoon (n=422)	Overall cohort – follow-up (n=453)	p-value†
11-deoxycortisol (pmol/L)	542 (269 - 1041)	462.8 (294 - 707.2)	465.7 (291 - 730)	0.560
11-deoxycortisol/cortisol (10^{-3})	2.38 (0.98 - 3.45)	2.33 (1.40 - 3.78)	2.33 (1.39 - 3.76)	0.361
Aldosterone (pmol/L)	132.3 (61.4 - 270.6)	143.7 (79.6 - 267)	143 (76.7 - 267)	0.580
PRC (mIU/L)	52 (9.0 - 265)	94.4 (29.9 - 344.8)	92.8 (26.1 - 327.8)	0.083
Aldosterone/PRC	1.63 (0.58 - 6.96)	1.50 (0.37 - 4.98)	1.51 (0.39 - 5.29)	0.303
Haemoglobin (g/dl)	12.3 (11.1 - 13.7)	12.5 (11.3 - 13.6)	12.5 (11.2 - 13.6)	0.952
Cardiovascular medication				
Diuretic	27 (87)	418 (99.1)	445 (98.2)	- ¥
ACE inhibitor	25 (81)	305 (72.3)	330 (72.9)	0.312
ACE inhibitor or ARB	28 (90)	335 (79.4)	363 (80.1)	0.141
Beta blocker	21 (68)	288 (68.3)	309 (68.2)	0.954
Aldosterone antagonist	7 (22.5)	57 (13.5)	64 (14.1)	0.162
Digoxin	6 (19)	109 (25.8)	115 (25.4)	0.424
Anti-arrhythmic	1 (3)	25 (5.9)	26 (5.7)	0.533
Aspirin	18 (58)	235 (55.7)	253 (55.9)	0.797
Statin	20 (64.5)	315 (74.6)	335 (74)	0.215
Non-cardiovascular medication				
Steroid tablets	1 (3.2)	13 (3.1)	14 (3.1)	- ¥

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)
† Patients who had blood sampling in the morning vs patients who had blood sampling in the afternoon during follow-up, Mann-Whitney test for continuous and χ^2 test for categorical variables
¥ Chi-Square approximation probably invalid

The levels of glucocorticoids, the physiological and laboratory measurements in patients who had blood samples taken in the morning during hospital admission and at follow-up and the medication prior to admission and after discharge are presented in Table 5-5.

Cortisol and 11-deoxycortisol levels were not significantly different but the 11-deoxycortisol to cortisol ratio was higher at follow-up compared with the hospital admission.

The majority of patients were in NYHA functional class III during hospital admission and in NYHA functional class II at follow-up. The weight, pulse rate, SBP and BNP were lower and the PRC was higher at the follow-up visit compared with hospital admission. A higher proportion of patients were taking an ACE inhibitor or ARB, a beta-blocker or a diuretic after discharge.

Table 5-5. Clinical characteristics, physiological and laboratory measurements and medication in patients who had blood samples taken in the morning during hospital admission and at follow-up (n=31)

Variable	During admission (n=31)	At follow-up (n=31)	p-value†
NYHA class			
I	0 (0)	1 (3)	- ¥
II	6 (19)	19 (61)	**0.004**
III	17 ((55)	11 (35.5)	0.210
IV	8 (26)	0 (0)	- ¥
Physiological measurements			
BMI (kg/m^2)	26.8 (24.5 - 33.7)	25.6 (23.9 - 30.9)	**0.001**
Weight (kg)	74.3 (65 - 96)	70 (63 - 88)	**0.001**
Pulse rate (bpm)	90 (68 - 110)	74 (62 - 86)	**0.005**
SBP (mmHg)	130 (110 - 161)	125 (107 - 140)	**0.029**
DBP (mmHg)	70 (60 - 74)	68 (56 - 75)	0.060
Laboratory measurements (blood)			
BNP (pg/ml)	1146 (337 - 1917)	423 (187 - 1015)	**<0.001**
Sodium (mmol/L)	139 (135 - 141)	140 (138 - 141)	0.177
Potassium (mmol/L)	4.2 (3.8 - 4.5)	4.2 (3.8 - 4.4)	0.187
Urea (mmol/L)	8.2 (5.8 - 10.1)	8.6 (6.4 - 11.6)	0.493
Creatinine (µmol/L)	109 (91 - 137)	110 (87 - 137)	0.468
eGFR (ml/min/1.73m^2)	57 (45 - 60)	60 (44 - 60)	0.888
eGFR <60ml/min/1.73m^2	17 (55)	15 (48)	0.688
Cholesterol (total) (mmol/L)	3.6 (2.9 - 4.3)	3.8 (3.2 - 4.9)	0.105
HDL (mmol/L)	0.9 (0.7 - 1.3)	1.0 (0.8 - 1.1)	0.587
CRP (mg/L)	14 (7.6 - 32)	5.9 (1.8 - 13)	**0.001**
TSH (mIU/L)	1.8 (1.4 - 2.5).	1.6 (0.9 - 2.3)	0.879
Cortisol (nmol/L)	281.8 (211.1 - 456.4)	274.5 (170.4 - 374.4)	0.637
11-deoxycortisol (pmol/L)	455.7 (231 - 781.3)	542 (269 - 1041)	0.218
11-deoxycortisol/cortisol (10^{-3})	1.61 (0.86 - 2.34)	2.38 (0.98 - 3.45)	**0.035**
Aldosterone (pmol/L)	68.9 (16.4 - 216.6)	132.3 (61.4 - 270.6)	0.076
PRC (mIU/L)	48 (15 - 103)	52 (9.0 - 265)	**0.050**
Aldosterone/PRC	1.68 (0.06 - 3.34)	1.63 (0.58 - 6.96)	0.623
Haemoglobin (g/dl)	12.5 (11 - 13.9)	12.3 (11.1 - 13.7)	0.380
Cardiovascular medication*			
Diuretic	19 (61)	27 (87)	**0.008**
ACE inhibitor	17 (55)	25 (81)	**0.008**

Variable	During admission (n=31)	At follow-up (n=31)	p-value†
ACE inhibitor or ARB	21 (68)	28 (90)	**0.016**
Beta-blocker	13 (42)	21 (68)	**0.008**
Aldosterone antagonist	3 (10)	7 (22.5)	0.219
Digoxin	4 (13)	6 (19)	0.625
Anti-arrhythmic	2 (6)	1 (3)	1
Aspirin	16 (52)	18 (58)	0.625
Statin	19 (61)	20 (64.5)	1
Non-cardiovascular medication			
Steroid tables	1 (3.2)	1 (3.2)	1

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).

† Wilcoxon matched pairs test was used for continuous variables and McNemars's test was used for categorical variables.

* medication prior to hospital admission for patients studied during admission

¥ Chi-Square approximation probably invalid

5.4 Discussion

5.4.1 Baseline patient characteristics at study visit

Just above three-fifths of the patients enrolled in the study during hospital admission returned for the follow-up visit, whilst almost a fifth of patients withdrew from the study following discharge from hospital due to refusal to participate. A similar proportion died or had deterioration in their health status prior to the follow-up visit, with the former subgroup consisting of more patients than the latter subgroup. The fact that a significant proportion of patients died prior to follow-up visit is in keeping with previous data showing an increased risk of in-hospital and post-discharge mortality in patients hospitalised with decompensated HF (345) (346). Hospitalised patients with worsening HF and worse short-term mortality or morbidity are likely to be older, have higher NYHA class and lower levels of sodium among other adverse prognostic markers in HF (347) (345) (346). In the current study, failure of patients to attend for the follow-up visit due to deterioration in health or death might have contributed to the improved patient characteristics seen during follow-up.

In this chapter, I saw the anticipated changes in the clinical status and laboratory measurements between hospital admission and the follow-up visit. The study visit cohort represents a population of patients with predominantly stable HF in comparison to patients with decompensated HF during hospital admission. Patients at follow-up had lower body weight than during hospital admission, likely due to extracellular fluid volume reduction following diuretic treatment. BNP levels were lower, with the median BNP value after discharge being less than half of the median BNP during hospitalisation. Similarly, CRP levels and the proportion of patients with elevated troponin were lower at the follow-up visit. Patients had similar urea and creatinine levels at both stages; however, the eGFR was higher in the post-discharge compared to the hospitalised cohort.

Nearly all patients were taking a diuretic at the follow-up visit. Although a higher proportion of patients were taking an ACE inhibitor/ARB or beta-blocker at follow-up compared with hospital admission, a considerable proportion was still not receiving an ACE inhibitor or ARB (20%) or a beta-blocker (32%) after discharge. That may be because patients could not tolerate these treatments, due to other comorbidities e.g. renal dysfunction, reversible airways disease. Finally, although the aldosterone blocker use increased after discharge compared with hospital admission, still a surprisingly small proportion of patients were treated with these agents (only 14%).

5.4.2 Levels of RAAS mediators during follow-up

Aldosterone levels during follow-up were well within the normal range (median 143 pmol/L, IQR [76.7 - 267]). In Val-HeFT, the median (IQR) aldosterone concentration in patients with HFrSF was 280 (166.4 - 471.6) pmol/L (285). In ALOFT and in another single centre study, the median (IQR) aldosterone in patients with HFrSF and HFpSF was 222 (98.3 - 418.3) pmol/L and 277 (155.3 - 529.8) pmol/L respectively (72) (348). The reasons for the differences in aldosterone levels between these studies and the current study are likely to be multifactorial. Almost all my patients (98.2%) were treated with a diuretic at the follow-up visit and any difference in the diuretic use between the aforementioned studies is unlikely to account for the lower aldosterone levels in my patients. As discussed previously (section 4.4.2), the study of RAAS and comparisons between different studies in terms of RAAS activity in patients taking a RAAS blocker is difficult. In the current study, 80% of patients were treated with an ACE inhibitor or ARB at follow-up compared with 99% of patients in ALOFT and 82% of patients in the single-centre study. ACE inhibitors and ARBs reduce aldosterone levels and the lower prescription rate of these agents in my patients, is unlikely to account for the lower levels of aldosterone. Aldosterone antagonists were used only by 14% of patients in the current study compared with 34% and 28.3% of patients in the other two

studies respectively. Aldosterone blockers increase aldosterone levels and the lower prescription rate of aldosterone blockers in the current study might contribute to the lower aldosterone levels I observed. Indeed, as shown in Table 5-2, aldosterone levels were markedly higher in patients taking an aldosterone blocker compared with patients not taking an aldosterone blocker. Alternatively, differences in terms of dosage of diuretics or RAAS inhibitors may additionally account for the differences in aldosterone levels; however, such information is not available.

One other possibility for the lower mineralocorticoid levels seen in the current study is the different assays used. To my knowledge, this is the first study to report on aldosterone (and other corticosteroid) levels, which were measured by LCMS in patients with HF. Aldosterone was measured by immunoassays in the previous HF studies. The levels of aldosterone in plasma are in the picomolar range and immunoassays are often inaccurate, especially at low normal concentrations. Plasma aldosterone levels were reported to be on average 33% higher when measured by a commercial radioimmunoassay compared with LCMS in the same blood samples (349). Moreover, immunoassays are susceptible to interference by cross-reacting corticosteroids, potentially giving consequently falsely high results (321). Marked differences, approaching even 100%, were previously reported when aldosterone levels measured by different immunoassays (322). The high accuracy and specificity of LCMS assay over the immunoassays with regards to aldosterone measurements has been increasingly recognised (323) and might at least partially account for the lower aldosterone levels observed in this study.

As previously discussed, aldosterone levels were higher in patients taking an aldosterone blocker; that was evident even in patients taking background therapy with an ACE inhibitor or ARB (Table 5-2). Aldosterone, apart from the activation of the MRs, exerts MR-independent

effects. Thus, higher levels of mineralocorticoids might be partially associated with worse cardiovascular effects due to their nongenomic actions. Inhibition of aldosterone synthase has been shown to decrease aldosterone levels; in patients with primary hyperaldosteronism, aldosterone synthase inhibitors decrease plasma aldosterone levels (350). Similar results with lowering of aldosterone levels following inhibition of aldosterone synthase were also reported in patients with essential hypertension (351). Thus, aldosterone synthase inhibitors might prevent the reactive increase in aldosterone levels in response to aldosterone blockers. That may provide further therapeutic benefit in combination with RAAS inhibitors in patients with HF.

PRC was raised in the overall cohort at follow-up and was higher compared with admission levels. PRC in this study was markedly higher than PRC measured in healthy subjects by using the same assay (352). Similarly, PRC levels in my patients were higher than the PRC reported in patients with hypertension receiving antihypertensive treatment again measured using the same assay (352). RAAS is one of the main neurohumoral pathways activated in HF and that is likely to account for the differences in PRC between HF patients in the current study and healthy subjects or patients with hypertension. Moreover, treatment with a diuretic or RAAS inhibitor which both increase PRC, potentially contributes to the differences in PRC at the aforementioned studies. Furthermore, the higher prescription rate of a diuretic or RAAS inhibitor is also likely to contribute to the higher PRC levels observed at follow-up visit compared with hospital admission.

The majority of previous studies in patients with HF analysed the PRA instead of PRC (353) (354). PRA refers to the rate of angiotensin I generation from angiotensinogen and is predominantly measured by radio-immunological assays. A significant correlation between

PRC and PRA has been recently reported in HF patients (354). Similar to the elevated PRA found in previous HF studies, PRC was raised in patients with chronic HF in my study.

Finally, PRC levels were higher in patients taking a RAAS inhibitor compared with those not taking a RAAS inhibitor as displayed in Table 5-2. That is of clinical importance, as higher renin secretion in these patients might overcome RAAS inhibition and in turn lead to higher levels of RAAS downstream components. Indeed, as shown in Table 13-17 & Table 13-18 in the Appendix, aldosterone levels in the overall cohort at follow-up were higher in patients with higher PRC. Similarly, PRC was higher in patients with higher aldosterone levels. Thus, in the overall post-discharge cohort, renin continues to drive aldosterone secretion, likely through RAAS mediators despite the treatment with an ACE inhibitor/ARB or an aldosterone blocker. The above findings indicate that aldosterone escape observed in patients with chronic HF, could be partially attributed to greater activation of upstream components of the RAAS, which overcome the RAAS inhibition in later steps. They furthermore imply that renin inhibition can potentially be a therapeutic option in order to suppress aldosterone escape. Indeed, in the ALOFT study, treatment with the direct renin inhibitor aliskiren resulted in reduction of urinary aldosterone secretion in patients with chronic HF (348).

5.4.3 Levels of glucocorticoids at follow-up

Levels of plasma cortisol in patients with stable HF were well within the normal range and lower than during admission. Glucocorticoid secretion exhibits a diurnal rhythm and this is likely to contribute to the difference in cortisol levels between admission and follow-up, as the blood samples were predominantly taken in the afternoon at follow-up and exclusively in the morning during admission. Indeed, cortisol levels were higher in the small group of patients who had blood samples collected in the morning compared with the majority of patients who had blood samples collected in the afternoon at the follow-up visit. This is in

contrast to previous studies (70) suggesting that the circadian rhythm in glucocorticoid secretion is present in patients with HF.

In order to examine further if the improvement in the clinical status contributed to the lower glucocorticoid levels after discharge, I compared the glucocorticoid levels at follow-up and admission in the subset of patients who had blood samples collected in the morning both on admission and at follow-up. Interestingly, no significant difference was present in cortisol levels at the two time points, indicating that the lower cortisol levels found at follow-up are probably due to the circadian rhythm. Nevertheless, although the levels of the cortisol were not different, a lower 11-deoxycortisol to cortisol ratio was found during admission compared with follow-up. It is accepted that lower 11-deoxycortisol to cortisol ratio is likely to reflect a higher activity of 11beta-hydroxylase, which converts the precursor 11-deoxycortisol to the end product cortisol (section 8.4.1). 11beta-hydroxylase is an ACTH dependent enzyme, and a lower 11-deoxycortisol to cortisol ratio indicates relatively higher HPA activity, however, that was not translated in higher levels of cortisol during admission.
Overall, similar to hospital admission no major activation of HPA axis was present in patients studied 4 to 6 weeks after discharge.

Glucocorticoid secretion at the follow-up visit will be further described in relation to RAAS mediators and other markers of HF severity in chapter 9.

6. Chapter Six - PRC and aldosterone levels during hospital admission in patients not taking a RAAS blocker

6.1 Introduction

Activation of the RAAS is thought to be fundamentally important in the pathophysiology of HF (25) (355). While there are many studies reporting RAAS activity in patients with decompensated HF, most of these included patients treated with some combination of an ACE inhibitor/ARB or aldosterone blocker (55) (339) (340). Treatment with a RAAS inhibitor affects the levels of RAAS components and this makes the interpretation of RAAS activity difficult. ACE inhibitors decrease the levels of angiotensin II and aldosterone and increase the levels of renin. ARBs eliminate the effects of angiotensin II, while they suppress the levels of aldosterone and stimulate the secretion of renin. Aldosterone blockers antagonise the aldosterone effects and increase both aldosterone and renin levels. Thus, in the presence of RAAS inhibitors the relationship between plasma components of RAAS, as well as the relationship between these mediators and the activation of their receptors, becomes distorted making consequently the study of RAAS activity in these patients difficult.

There are few available data on RAAS activity in patients with decompensated HF not treated with a RAAS inhibitor. The few studies that do exist included only small number of patients and do not report consistent findings (57) (58) (60). The main purpose of this chapter is to describe RAAS activity in patients with decompensated HF and who were not treated with an ACE inhibitor/ARB or aldosterone blocker prior to hospitalisation. The markers of RAAS activity measured were plasma aldosterone and PRC. In addition, the aldosterone to PRC ratio was calculated. In this chapter, I also present the clinical characteristics of my patients according to RAAS activity.

6.2 Methods

6.2.1 Study participants and laboratory measurements

Details of the study participants and the laboratory measurements were presented previously in sections 2.3.1 & 2.3.2. Only patients not receiving a RAAS inhibitor (ACE inhibitor/ARB or aldosterone blocker) prior to hospital admission were included in the current study.

6.2.2 Statistical analysis

All baseline characteristics were expressed as median (IQR) for continuous and as absolute number (percentage) for categorical variables. The inter-group comparisons were carried out by the Mann-Whitney and Kruskal-Wallis test for continuous variables as appropriate and by the χ^2 test for categorical variables. A p-value <0.05 was considered significant for all analyses. Statistical analyses were performed with Minitab version 15.

6.3 Results

6.3.1 Patient characteristics during hospital admission stratified by treatment with a RAAS inhibitor

Of the 722 patients enrolled, 278 received none of an ACE inhibitor/ARB or aldosterone blocker prior to hospital admission (Table 6-1). Patients not taking a RAAS inhibitor were more often women and were less likely to have a history of previous HF, MI, angina, diabetes or hypertension compared with patients taking a RAAS inhibitor and patients of the overall hospitalised cohort. The weight, urea and creatinine were significantly lower and the pulse rate, SBP and DBP, haemoglobin and cholesterol were significantly higher in the first group compared with the other two groups. Diuretics, beta-blockers, aspirin and statins were less often prescribed in patients not taking a RAAS inhibitor prior to admission.

Table 6-1. Patient characteristics during hospital admission stratified by treatment with a RAAS inhibitor*

Variable	Patients not taking a RAAS inhibitor (n=278)	Patients taking a RAAS inhibitor (n=444)	All patients (n=722)	p-value¶	p-value†
Age (years)	75 (67 - 82)	74 (68 – 80)	74 (68 - 81)	0.378	0.557
Female gender	146 (52.5)	186 (41.9)	332 (46)	**0.005**	0.064
NYHA class					
II	72 (25.9)	102 (23)	174 (24.1)	0.371	0.554
III	166 (59.7)	269 (60.6)	435 (60.3)	0.815	0.877
IV	40 (14.4)	73 (16.4)	113 (15.7)	0.460	0.619
Medical history					
HF	73 (26.3)	247 (55.6)	320 (44.3)	**<0.001**	**<0.001**
MI	92 (33.1)	230 (51.8)	322 (44.6)	**<0.001**	**0.001**
Angina	120 (43.2)	276 (62.2)	396 (54.8)	**<0.001**	**0.001**
Diabetes mellitus	48 (17.3)	179 (40.3)	227 (31.4)	**<0.001**	**<0.001**
Hypertension	159 (57.2)	319 (71.9)	478 (66.2)	**<0.001**	**0.008**
AF	142 (51.1)	245 (55.2)	387 (53.6)	0.282	0.474
CVA/TIA	55 (19.8)	100 (22.5)	155 (21.5)	0.383	0.558
Physiological measurements					
BMI (kg/m^2)	27.1 (22.6 – 31.8)	28.6 (24.9 - 34.1)	27.9 (24 – 32.9)	**<0.001**	**0.007**
Weight (kg)	71.1 (58.9 – 87.1)	78 (65 – 92)	76 (62.2 - 90)	**<0.001**	**0.006**
Pulse rate (bpm)	94 (74.8 – 110)	82 (70 - 100)	86 (71.8 - 106)	**<0.001**	**0.002**

Variable	Patients not taking a RAAS inhibitor (n=278)	Patients taking a RAAS inhibitor (n=444)	All patients (n=722)	p-value¶	p-value†
SBP (mmHg)	137 (119 – 155)	130 (114 - 150)	134 (115 – 152)	**0.001**	**0.030**
DBP (mmHg)	80 (66 - 92)	71.5 (60 - 85)	75 (62 - 88)	**<0.001**	**0.003**
Signs of fluid congestion					
Elevated JVP	193 (78.1)	319 (80.2)	512 (79.4)	0.539	0.683
Peripheral oedema	201 (72.3)	341 (76.8)	542 (75.1)	0.174	0.370
ECG rhythm					
SR	156 (56.1)	242 (54.5)	398 (55.1)	0.672	0.778
AF	113 (40.7)	181 (40.8)	294 (40.7)	0.975	0.983
Echocardiography measurements					
LVEDD (cm)	5.1 (4.5 - 5.8)	5.2 (4.7 - 6.0)	5.2 (4.6 - 5.9)	**0.033**	0.082
Dilated left ventricle	76 (33.8)	115 (40.1)	191 (37.3)	0.144	0.359
LVH	99 (44.2)	127 (44.6)	226 (44.4)	0.934	0.959
LVSD	153 (68)	188 (65.5)	341 (66.6)	0.552	0.710
Laboratory measurements (blood)					
BNP (pg/ml)	886 (419 - 1955)	859 (369 - 1746)	871 (391 - 1819)	0.196	0.388
Troponin I ≥ 0.04 (µg/L)**	131 (57.5)	199 (53.8)	330 (55.1)	0.380	0.557
Sodium (mmol/L)	138 (135 – 141)	138 (135 - 141)	138 (135 - 141)	0.908	0.939
Potassium (mmol/L)	4.1 (3.8 - 4.5)	4.2 (3.8 - 4.5)	4.2 (3.8 – 4.5)	0.299	0.489
Urea (mmol/L)	7.9 (6.0 - 10.7)	9.5 (6.6 – 13.2)	8.7 (6.3 - 12)	**<0.001**	**0.008**

Variable	Patients not taking a RAAS inhibitor (n=278)	Patients taking a RAAS inhibitor (n=444)	All patients (n=722)	p-value¶	p-value†
Creatinine (µmol/L)	100 (82 - 127.3)	111 (88 – 141.8)	106.5 (85 - 137)	**0.001**	**0.025**
eGFR (ml/min/1.73m^2)	59 (44 - 60)	55 (40 – 60)	56 (41- 60)	**0.034**	0.158
eGFR <60ml/min/1.73m^2	143 (51.4)	262 (59)	405 (56.1)	**0.046**	0.185
Cholesterol (total) (mmol/L)	4.1 (3.4 - 4.9)	3.6 (3.0 - 4.3)	3.7 (3.1 – 4.6)	**<0.001**	**0.003**
HDL (mmol/L)	1.0 (0.8 - 1.3)	1.0 (0.8 - 1.3)	1.0 (0.8 – 1.3)	0.184	0.371
CRP (mg/L)	13 (6.1 - 32.5)	13 (5.5 - 31)	13 (5.7 - 32)	0.381	0.559
TSH (mIU/L)	1.8 (1.2 - 2.8)	1.7 (1.0 - 2.8)	1.7 (1.0 - 2.8)	0.289	0.477
Haemoglobin (g/dl)	12.5 (10.8 – 14)	11.9 (10.4 - 13.2)	12.1 (10.6 - 13.5)	**0.002**	**0.037**
Cardiovascular medication					
Diuretic	144 (51.8)	354 (79.7)	498 (69)	**<0.001**	**<0.001**
Beta-blocker	102 (36.7)	244 (55)	346 (47.9)	**<0.001**	**0.001**
Digoxin	34 (12.2)	83 (18.7)	117 (16.2)	**0.022**	0.116
Anti-arrhythmic	11 (4.0)	18 (4.1)	29 (4.0)	0.948	0.966
Aspirin	132 (47.5)	256 (57.7)	388 (53.7)	**0.008**	0.076
Statin	133 (47.8)	338 (76.1)	471 (65.2)	**<0.001**	**<0.001**

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)
***** ACE inhibitor/ARB or aldosterone blocker
¶ Patients not taking a RAAS inhibitor (n=278) vs patients taking a RAAS inhibitor (n=444), Mann-Whitney test for continuous and χ^2 test for categorical variables.
† Patients not taking a RAAS inhibitor (n=278) vs the overall hospitalised cohort (n=722), Mann-Whitney test for continuous and χ^2 test for categorical variables
****** measured at WIG and GRI

6.3.2 RAAS activity during hospital admission

Levels of plasma aldosterone and PRC, and the aldosterone to PRC ratio, during hospital admission are presented below.

6.3.2.1 Aldosterone

An aldosterone level measured during hospital admission was available in 210 of the 278 patients. The median (IQR) aldosterone was 80.3 (41.8 - 184.7) pmol/L (Figure 6-1) and the mean (SD) aldosterone was 184.3 (421.5) pmol/L. A frequency distribution histogram of aldosterone levels during hospital admission in these patients is displayed in Figure 6-2. The distribution of aldosterone concentrations was positively skewed. The minimum aldosterone concentration was 2.5 pmol/L and maximum aldosterone concentration was 5399 pmol/L. Almost all patients (98%) had aldosterone levels within the normal range (0 - 937 pmol/L).

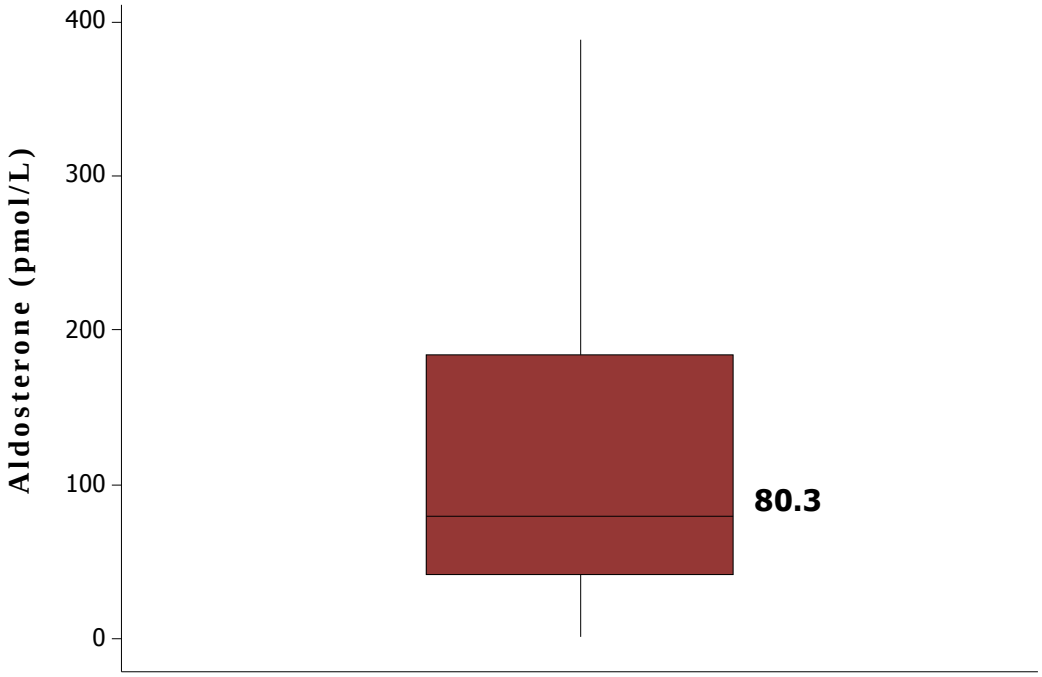


Figure 6-1. Box and whisker plot of the aldosterone concentrations showing the 2.5, 25, 50, 75 and 97.5 centiles

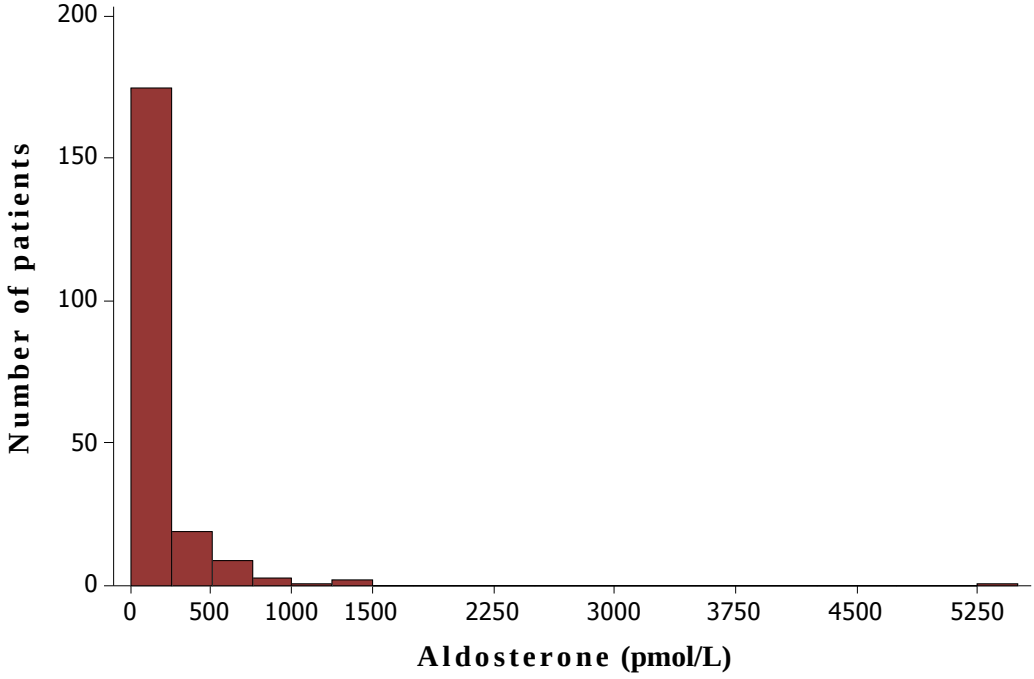


Figure 6-2. Frequency distribution histogram of aldosterone levels in patients not receiving an ACE inhibitor/ARB or aldosterone blocker

6.3.2.2 PRC

A PRC measured during hospital admission was available in 267 of the 278 patients. The median (IQR) PRC was 27.2 (9.2 - 78.6) mIU/L (Figure 6-3) and the mean (SD) PRC was 115.8 (371.7) mIU/L. A frequency distribution histogram of PRC levels during hospital admission in these patients is displayed in Figure 6-4. The distribution of PRC values was positively skewed. The minimum PRC was 5.0 mIU/L and the maximum PRC was 4898 mIU/L. Sixty-two percent of patients had PRC within the normal range (5.0 - 44.9 mIU/L).

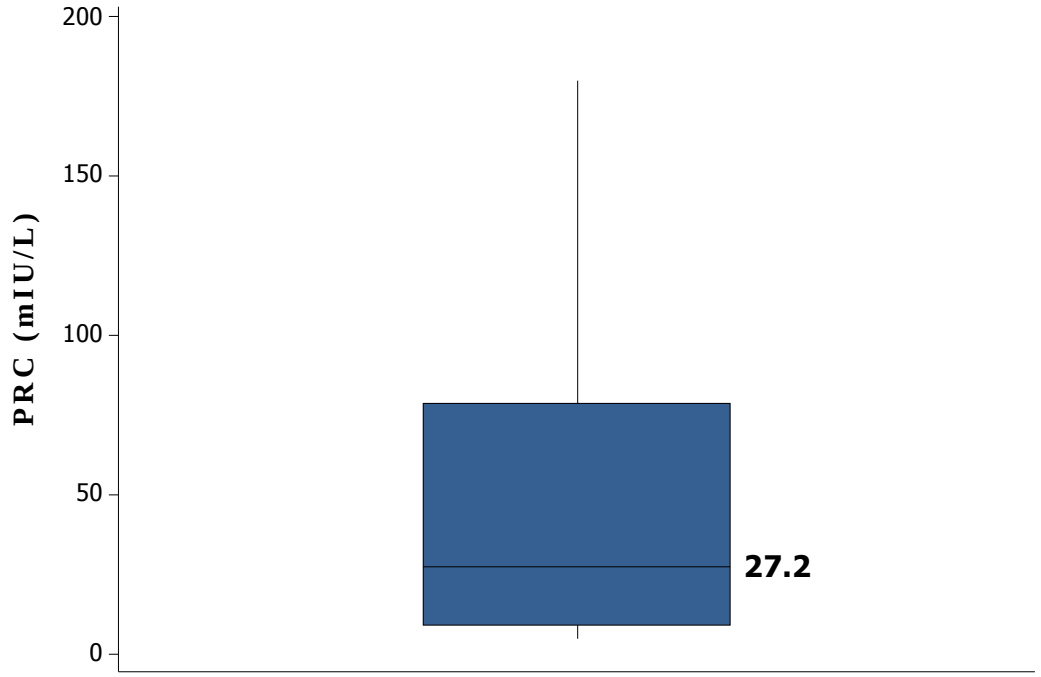


Figure 6-3. Box and whisker plot of PRC values showing the 2.5, 25, 50, 75 and 97.5 centiles

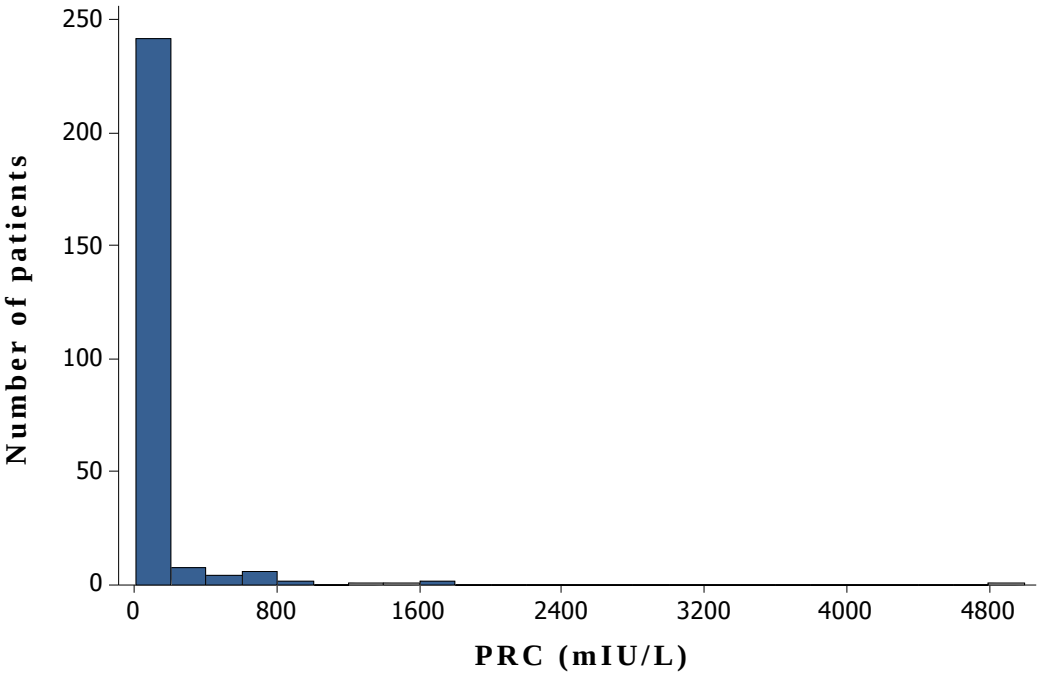


Figure 6-4. Frequency distribution histogram of PRC in patients not receiving an ACE inhibitor/ARB or aldosterone blocker

6.3.2.3 Aldosterone to PRC ratio

An aldosterone to PRC ratio could be calculated for 207 of the 278 patients. The median (IQR) aldosterone to PRC ratio was 3.11 (1.09 - 7.51) (Figure 6-5) and the mean (SD) aldosterone to PRC was 6.9 (14). A frequency distribution histogram of aldosterone to PRC levels during hospital admission in these patients is displayed in Figure 6-6. The distribution of aldosterone to PRC values was positively skewed. The minimum aldosterone to PRC ratio was 0.03 and the maximum aldosterone to PRC ratio was 140.1.

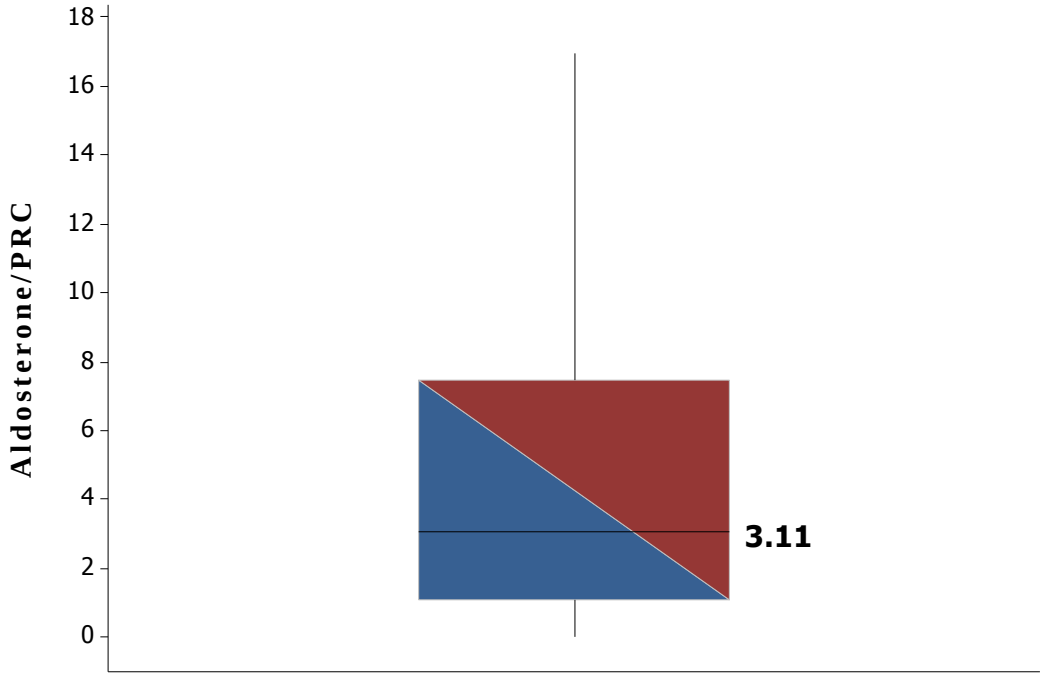


Figure 6-5. Box and whisker plot of the aldosterone to PRC ratio showing the 2.5, 25, 50, 75 and 97.5 centiles

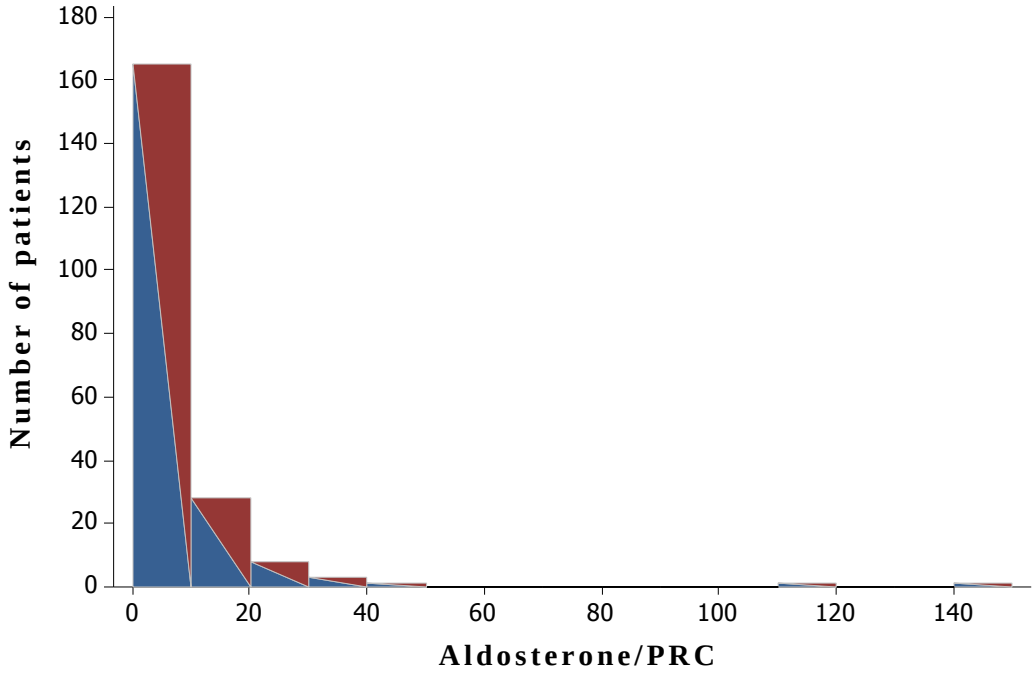


Figure 6-6. Frequency histogram of aldosterone to PRC ratio in patients not receiving ACE inhibitor/ARB or aldosterone blocker

6.3.3 Patient characteristics according to RAAS activity

The characteristics of the 278 patients not taking a RAAS inhibitor were stratified according to the levels of aldosterone and PRC and the aldosterone to PRC ratio during the hospital admission.

6.3.3.1 Patient characteristics according to aldosterone levels

The group of 210 patients with measured aldosterone levels was divided into 4 subgroups, according to the median aldosterone and the 25^{th} and 75^{th} centiles. Such quartiles were respectively defined by aldosterone levels <41.8 pmol/L, 41.8 to 80.2 pmol/L, 80.3 to 184.6 pmol/L and ≥ 184.7 pmol/L (Table 6-2).

Compared with those in the lowest aldosterone quartile, participants in the highest quartile were more likely to be younger and female. Patients in the highest aldosterone quartile also had higher PRC and higher aldosterone to PRC ratio. Apart from the above differences, a trend for higher cortisol was also present in patients with higher aldosterone levels.

Table 6-2. Characteristics of patients not taking an ACE inhibitor/ARB or aldosterone blocker according to aldosterone quartiles

Variable	Q1 (n=52)	Q2 (n=53)	Q3 (n=53)	Q4 (n=52)	p-value†
Age (years)	75 (62 – 80)	78 (72 – 86)	77 (69 – 85)	69 (65 – 81)	**0.004**
Female gender	19 (36.5)	29 (54.7)	32 (60.4)	31 (59.6)	0.051
NYHA class					
II	17 (32.7)	12 (22.6)	16 (30.2)	14 (26.9)	0.688
III	28 (53.9)	36 (67.9)	31 (58.5)	29 (55.8)	0.467
IV	7 (13.5)	5 (9.4)	6 (11.3)	9 (17.3)	0.659
Medical history					
HF	12 (23.1)	10 (18.9)	20 (37.7)	16 (30.8)	0.136
MI	16 (30.8)	18 (34)	17 (32.1)	16 (30.8)	0.983
Angina	18 (34.6)	28 (52.8)	24 (45.3)	21 (40.4)	0.281
Diabetes mellitus	12 (23.1)	12 (22.6)	8 (15.1)	9 (17.3)	0.666
Hypertension	31 (59.6)	31 (58.5)	33 (62.3)	28 (53.9)	0.850
AF	30 (57.7)	25 (47.2)	30 (56.6)	27 (51.9)	0.688
CVA/TIA	12 (23.1)	15 (28.3)	8 (15.1)	8 (15.4)	0.264
Physiological measurements					
BMI (kg/m^2)	27.0 (23.4 – 32.5)	24.4 (22.3 – 30.6)	28.2 (22.8 – 31.5)	28.3 (22.2 – 34.1)	0.508
Pulse rate (bpm)	99 (79 – 112)	92 (72 – 108)	96 (75 – 107)	88 (75 – 110)	0.757
SBP (mmHg)	140 (122 – 156)	138 (129 – 154)	126 (115 – 152)	134 (114 – 150)	0.231
DBP (mmHg)	80 (69 – 97)	82 (70 – 93)	77 (65 – 88)	80 (63 – 92)	0.539

Variable	Q1 (n=52)	Q2 (n=53)	Q3 (n=53)	Q4 (n=52)	p-value†
Signs of fluid congestion					
Elevated JVP	34 (72.2)	39 (83)	37 (75.4)	36 (80)	0.614
Peripheral oedema	44 (84.6)	36 (67.9)	40 (75.5)	34 (65.4)	0.113
ECG rhythm					
SR	26 (50)	34 (64.2)	25 (47.2)	29 (55.8)	0.309
AF	25 (48.1)	16 (30.2)	26 (49.1)	20 (38.5)	0.160
Echocardiography measurements					
LVEDD (cm)	5.2 (4.8 – 5.8)	5.2 (4.4 – 5.9)	5.0 (4.5 – 5.4)	5.3 (4.6 – 6.0)	0.325
Dilated left ventricle	14 (30.4)	18 (40)	12 (29.3)	17 (42.5)	0.482
LVH	20 (43.5)	22 (50)	21 (51.2)	15 (37.5)	0.571
LVSD	28 (60.9)	31 (68.9)	24 (58.5)	31 (77.5)	0.252
Laboratory measurements (blood)					
BNP (pg/ml)	826 (397 – 2138)	841 (358 – 1966)	827 (448 – 1530)	994.5 (407 – 2006)	0.980
Troponin I ≥ 0.04 (µg/L)*	29 (70.7)	26 (59.1)	24 (52.2)	24 (54.6)	0.311
Sodium (mmol/L)	138 (135 – 140)	138 (133 – 140)	139 (135 – 141)	138 (135.3 – 141)	0.547
Potassium (mmol/L)	4.1 (3.9 – 4.5)	4.2 (3.9 – 4.5)	4.2 (3.8 – 4.7)	4.1 (3.6 – 4.3)	0.164
Urea (mmol/L)	7.9 (6.0 – 11.7)	7.6 (6.1 – 10.3)	8.4 (5.9 – 11)	8.0 (6.3 – 11.7)	0.974
Creatinine (µmol/L)	101 (81 – 127)	97 (82 – 124)	115 (81 – 145)	105 (84 – 129)	0.499
eGFR (ml/min/1.73m^2)	60 (46 – 60)	60 (48 – 60)	49 (37 – 60)	59 (38 – 60)	0.232
eGFR <60ml/min/1.73m^2	25 (48.1)	25 (47.2)	33 (62.3)	29 (55.8)	0.361

Variable	Q1 (n=52)	Q2 (n=53)	Q3 (n=53)	Q4 (n=52)	p-value†
Cholesterol (total) (mmol/L)	3.7 (3.3 – 4.9)	4.6 (3.7 – 5.3)	3.8 (3.2 – 4.4)	4.1 (3.4 – 5.0)	0.059
HDL (mmol/L)	1.0 (0.8 – 1.3)	1.2 (1.0 – 1.7)	1.1 (0.9 – 1.4)	0.9 (0.8 – 1.2)	**0.050**
CRP (mg/L)	16.5 (6.6 – 45.5)	13.0 (5.8 – 35.0)	11.0 (5.6 – 24.5)	12.0 (7.0 – 40.0)	0.400
Cortisol (nmol/L)	325.1 (250.5 – 423.4)	359.4 (285.0 – 486.3)	322.6 (225.9 – 424.6)	403.0 (223.6 – 487.6)	0.166
11-deoxycortisol (pmol/L)	407.8 (255.9 – 801.7)	621.6 (315.0 – 1315.0)	513.0 (333.4 – 772.8)	555.3 (311.0 – 252.0)	0.127
11-deoxycortisol/cortisol (10^{-3})	1.35 (0.87 – 2.52)	2.17 (1.28 – 3.81)	1.65 (1.09 – 2.64)	1.54 (0.87 – 3.73)	0.079
PRC (mIU/L)	18.5 (5.0 – 67.1)	17.5 (7.3 – 53.8)	36.2 (12.0 – 66.8)	72.5 (23.0 – 151.0)	**<0.001**
Aldosterone/PRC	0.96 (0.32 – 3.31)	3.40 (1.29 – 7.51)	3.47 (1.81 – 10.7)	5.22 (2.56 – 14.0)	**<0.001**
TSH (mIU/L)	1.9 (1.0 – 4.0)	1.6 (1.2 – 1.9)	1.8 (0.9 – 2.7)	1.8 (1.5 – 3.1)	0.356
Haemoglobin (g/dl)	12.8 (11.6 – 14.5)	12.4 (10.5 – 13.7)	12.3 (11.2 – 13.8)	12.6 (10.4 – 14.0)	0.408
Cardiovascular medication prior to admission					
Diuretic	27 (51.9)	23 (43.4)	31 (58.5)	32 (61.5)	0.250
Beta-blocker	23 (44.2)	16 (30.2)	22 (41.5)	18 (34.6)	0.431
Digoxin	9 (17.3)	7 (13.2)	8 (15.1)	5 (9.6)	0.707
Anti-arrhythmic	1 (1.9)	3 (5.7)	3 (5.7)	3 (5.8)	0.746
Aspirin	26 (50)	27 (50.9)	30 (56.6)	20 (38.5)	0.303
Statin	26 (50)	22 (41.5)	28 (52.8)	25 (48.1)	0.689

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).
† Kruskal-Wallis test was used for continuous variables and χ^2 test for categorical variables.
¥ Chi-Square approximation probably invalid
*measured at WIG and GRI

6.3.3.2 Patient characteristics according to PRC levels

The group of 267 patients with measured PRC was divided into 4 subgroups, according to the median PRC and the 25^{th} and 75^{th} centiles. Such quartiles were respectively defined by PRC <9.2 mIU/L, 9.2 to 27.1 mIU/L, 27.2 to 78.5 mIU/L and ≥ 78.6 mIU/L (Table 6-3)

Compared with those in the lowest PRC quartile, participants in the highest PRC quartile were more likely to have lower aldosterone to PRC and 11-deoxycortisol to cortisol ratio and higher aldosterone, CRP and elevated troponin. They also had had lower SBP and higher urea. Patients with higher PRC were also more likely to be in sinus rhythm (SR) on the 12-lead ECG and less likely to have LVH on the transthoracic echocardiogram compared with patients with lower PRC, who were more likely to be in AF and have LVH on the transthoracic echocardiogram. In addition, a trend for lower DBP and sodium and higher creatinine and cortisol was present in patients with higher PRC. A trend for higher prevalence of dilated left ventricle and LVSD was also evident in these patients

.

Table 6-3. Characteristics of patients not receiving and ACE inhibitor/ARB or aldosterone blocker according to PRC quartiles

Variable	Q1 (n=66)	Q2 (n=67)	Q3 (n=67)	Q4 (n=67)	p-value†
Age (years)	77 (69 – 85)	75 (68 – 83)	75 (68 – 80)	70 (65 – 80)	0.127
Female gender	35 (53)	39 (58.2)	36 (53.7)	29 (43.3)	0.364
NYHA class					
II	19 (28.8)	16 (23.9)	26 (38.8)	10 (14.9)	**0.017**
III	37 (56.1)	43 (64.2)	35 (52.3)	43 (64.2)	0.393
IV	10 (15.2)	8 (11.9)	6 (9.0)	14 (20.9)	0.231
Medical history					
HF	14 (21.2)	15 (22.4)	21 (31.3)	18 (26.9)	0.522
MI	18 (27.3)	19 (28.4)	26 (38.8)	24 (35.8)	0.408
Angina	28 (42.4)	23 (34.3)	38 (56.7)	26 (38.8)	0.053
Diabetes mellitus	10 (15.2)	9 (13.4)	13 (19.4)	15 (22.4)	0.516
Hypertension	35 (53)	43 (64.2)	40 (59.7)	37 (55.2)	0.570
AF	41 (62.1)	42 (62.7)	31 (46.3)	26 (38.8)	**0.010**
CVA/TIA	15 (22.7)	16 (23.9)	13 (19.4)	10 (14.9)	0.571
Physiological measurements					
BMI (kg/m^2)	27.1 (23.3 – 33.3)	26.9 (22.6 - 30.7)	26.5 (22.2 – 31.9)	27p.8 (22.2 – 31.5)	0.780
Pulse rate (bpm)	91 (70 – 108)	92 (78 - 110)	92 (76 - 110)	98 (78 - 115)	0.698
SBP (mmHg)	140 (126 – 163)	141 (124 – 155)	136 (116 – 160)	127 (110 – 140)	**0.002**
DBP (mmHg)	81 (70 – 94)	80 (70 – 93)	80 (61 – 95)	74 (62 – 85)	0.070

Variable	Q1 (n=66)	Q2 (n=67)	Q3 (n=67)	Q4 (n=67)	p-value†
Signs of fluid congestion					
Elevated JVP	41 (75.9)	51 (78.5)	42 (75)	50 80.7)	0.882
Peripheral oedema	50 (75.7)	51 (76.1)	48 (71.6)	46 68.7)	0.731
ECG rhythm					
SR	31 (47)	30 (44.8)	38 (56.7)	48 (71.6)	**0.007**
AF	33 (50)	36 (53.7)	25 (37.3)	17 (25.4)	**0.003**
Echocardiography measurements					
LVEDD (cm)	5.1 (4.4 – 5.5)	5.0 (4.5 – 5.5)	5.0 (4.6 – 5.7)	5.5 (4.6 – 6.1)	0.150
Dilated left ventricle	14 (25.5)	16 (28.6)	17 (33.3)	25 (47.2)	0.084
LVH	32 (58.2)	27 (48.2)	19 (38)	17 (32.1)	**0.034**
LVSD	33 (60)	35 (62.5)	35 (68.6)	41 (77.4)	0.225
Laboratory measurements (blood)					
BNP (pg/ml)	838 (429 – 1650)	712 (417 – 1510)	906 (304 – 1715)	1189 (554 – 2174)	0.417
Troponin I ≥ 0.04 (µg/L)*	25 (44)	28 (48.3)	32 (57.1)	40 (80)	**0.001**
Sodium (mmol/L)	139 (137 – 140)	138 (135 – 141)	138 (135 – 141)	137 (135 – 140)	0.080
Potassium (mmol/L)	4.1 (3.8 – 4.5)	4.3 (3.9 – 4.6)	4.1 (3.9 – 4.4)	4.1 (3.7 – 4.5)	0.195
Urea (mmol/L)	7.3 (5.8 – 9.3)	7.6 (5.9 - 10.2)	8.5 (6.0 – 10.9)	8.8 (6.7 – 12.8)	**0.024**
Creatinine (µmol/L)	93 (78 – 115)	98 (80 – 121)	109 (82 – 144)	108 (87 – 133)	0.078
eGFR (ml/min/1.73m^2)	60 (46 – 60)	60 (46 – 60)	53 (38 – 60)	57 (43 – 60)	0.177
eGFR <60ml/min/1.73m^2	32 (48.5)	30 (44.8)	39 (58.2)	37 (55.2)	0.387

Variable	Q1 (n=66)	Q2 (n=67)	Q3 (n=67)	Q4 (n=67)	p-value†
Cholesterol (total) (mmol/L)	3.9 (3.5 – 4.8)	4.4 (3.2 – 5.0)	4.1 (3.4 – 4.8)	4.2 (3.3 – 5.0)	0.942
HDL (mmol/L)	1.1 (1.0 – 1.4)	1.1 (0.9 – 1.5)	1.0 (0.9 – 1.4)	0.9 (0.7 – 1.2)	**0.008**
CRP (mg/L)	8.2 (4.3 – 21.0)	16.0 (6.3 – 32.0)	13.0 (6.1 – 29.0)	18.0 (9.0 – 50.0)	**0.003**
Cortisol (nmol/L)	322.6 (229.9 – 428.6)	340.0 (219.7 – 475.5)	324.3 (253.2 – 452.2)	358.8 (279.0 - 485.0)	0.647
11-deoxycortisol (pmol/L)	509.6 (342.0 – 1213.0)	537.2 (340.0 – 978.0)	507.3 (281.4 – 910.4)	427.5 (230.0 – 911.0)	0.260
11-deoxycortisol/cortisol (10^{-3})	2.04 (1.38 – 3.15)	1.77 (1.18 - 3.33)	1.45 (0.83 – 2.73)	1.37 (0.76 – 2.42)	**0.027**
Aldosterone (pmol/L)	57.6 (26.1 – 118.3)	76.5 (36.4 – 130.9)	102.1 (60.3 – 223.7)	160.4 (54.0 – 412.0)	**<0.001**
Aldosterone/PRC	9.52 (5.0 – 17.7)	4.72 (2.63 – 7.57)	2.12 (1.24 – 3.61)	0.77 (0.25 – 2.05)	**<0.001**
TSH (mIU/L)	1.6 (1.0 – 2.5)	1.8 (1.3 – 3.1)	1.6 (1.0 – 2.6)	1.7 (1.4 – 3.0)	0.511
Haemoglobin (g/dl)	12.5 (11.3 – 14.1)	12.3 (11.2 – 13.6)	12.2 (10.4 – 14.2)	12.7 (10.3 – 13.9)	0.777
Cardiovascular Medication					
Diuretic	28 (42.4)	36 (53.7)	33 (49.3)	42 (62.7)	0.123
Beta-blocker	25 (37.9)	20 (29.8)	34 (50.8)	19 (28.4)	**0.028**
Digoxin	11 (16.7)	8 (11.9)	9 (13.4)	5 (7.5)	0.441
Anti-arrhythmic	4 (6.1)	1 (1.5)	3 (4.5)	3 (4.5)	0.606
Aspirin	34 (51.5)	27 (40.3)	41 (61.2)	26 (38.8)	**0.031**
Statin	28 (42.4)	33 (49.3)	38 (56.7)	29 (43.3)	0.321

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).
† Kruskal-Wallis test was used for continuous variables and χ^2 test for categorical variables.
¥ Chi-Square approximation probably invalid
*measured at WIG and GRI

Patients were further stratified in 4 groups according to the median PRC and SBP values. The levels of creatinine according to PRC and SBP are displayed in Figure 6-7 below. Patients with PRC above median and SBP above median had higher creatinine and patients with PRC below median and SBP above median had lower creatinine.

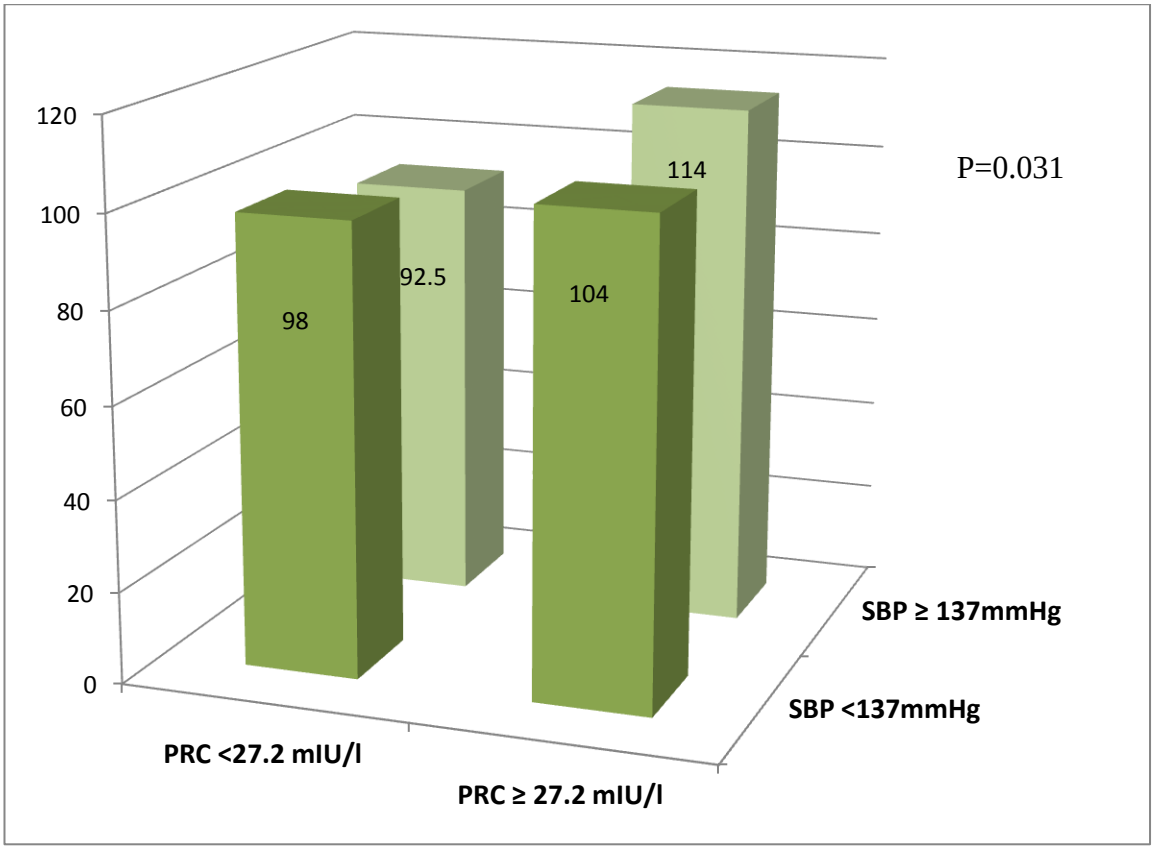


Figure 6-7. Levels of creatinine stratified by levels of PRC and SBP in patients not taking a RAAS inhibitor

The levels of eGFR according to PRC and SBP are displayed in Figure 6-8 below. Patients with PRC below median (with either SBP above or below median) had higher eGFR and patients with PRC above median and SBP below median had lower eGFR, however these differences did not reach statistical significance.

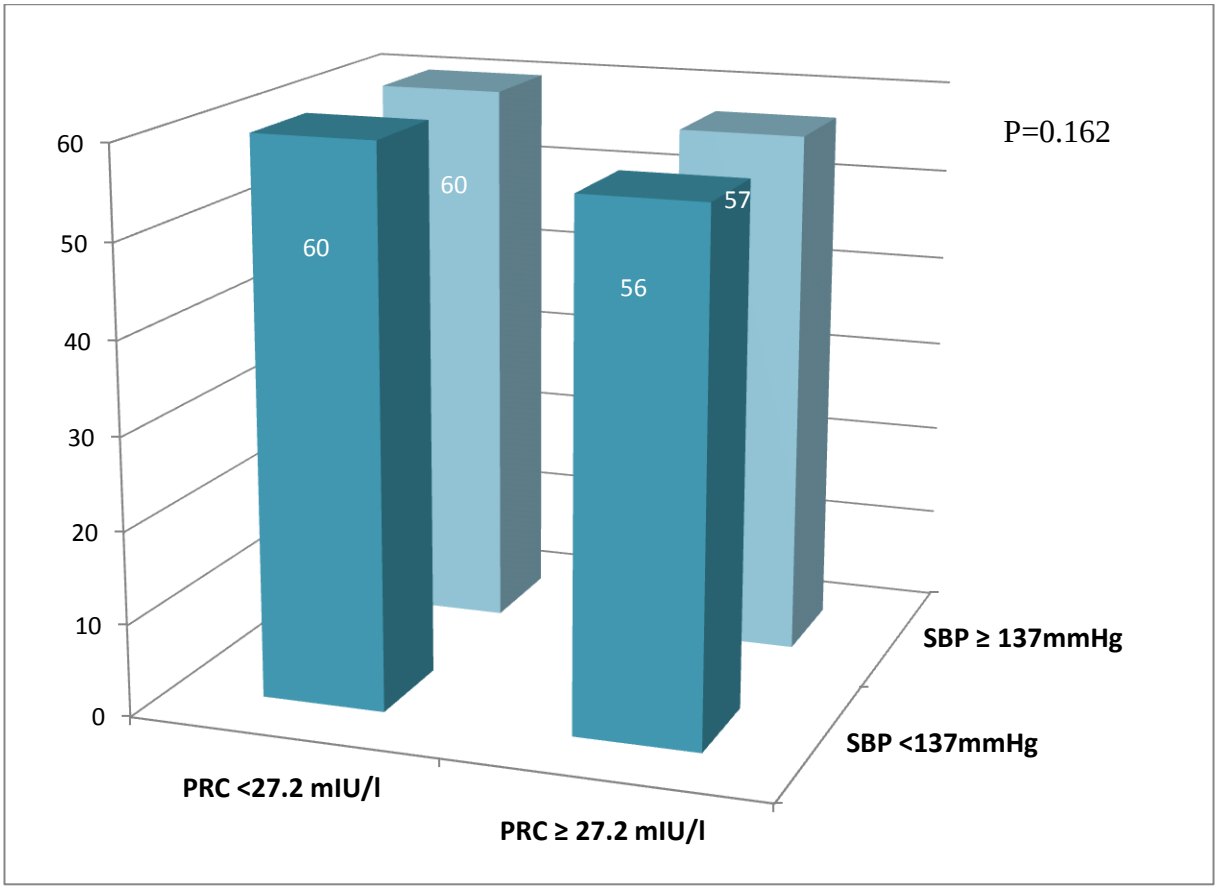


Figure 6-8. Levels of eGFR stratified by levels of PRC and SBP in patients not taking a RAAS inhibitor

6.3.3.3 Patient characteristics according to aldosterone to PRC ratio

The group of 207 patients was divided into 4 subgroups, according to the median aldosterone to PRC ratio and the 25^{th} and 75^{th} centiles. Such groups were respectively defined by aldosterone to PRC ratio <1.09, 1.09 to 3.10, 3.11 to 7.50 and ≥ 7.51 (Table 6-4).

Compared with those in the highest aldosterone to PRC ratio quartile, participants in the lowest aldosterone to PRC quartile were more likely to have higher PRC, CRP and elevated troponin. Patients with lower aldosterone to PRC ratio were also more likely to have lower aldosterone and sodium and 11-deoxycortisol to cortisol ratio compared with patients with higher aldosterone to PRC ratio. Moreover, a trend for lower SBP and DBP and higher urea and BNP and for lower prevalence of LVH on the transthoracic echocardiogram was evident in patients with lower aldosterone to PRC ratio.

Table 6-4. Characteristics of patients not taking an ACE inhibitor/ARB or aldosterone blocker according to aldosterone to PRC quartiles

Variable	Q1 (n=51)	Q2 (n=53)	Q3 (n=52)	Q4 (n=51)	p-value†
Age (years)	77 (68 - 82)	75 (67 – 83)	77 (68 – 84)	73 (67 – 83)	0.757
Female gender	22 (43.1)	24 (45.3)	34 (65.4)	30 (58.8)	0.067
NYHA class					
II	11 (21.6)	17 (32.1)	16 (30.8)	15 (29.4)	0.640
III	35 (68.6)	27 (51)	31 (59.6)	29 (56.7)	0.324
IV	5 (9.8)	9 (17)	5 (9.6)	7 (13.7)	0.622
Medical history					
HF	15 (29.4)	13 (24.5)	14 (26.9)	16 (31.4)	0.877
MI	18 (35.3)	21 (39.6)	14 (26.9)	13 (25.5)	0.350
Angina	21 (41.2)	25 (47.2)	21 (40.4)	23 (45.1)	0.882
Diabetes mellitus	15 (29.4)	8 (15.1)	9 (17.3)	9 (17.7)	0.255
Hypertension	31 (60.8)	28 (52.8)	30 (57.7)	33 (64.7)	0.654
AF	27 (52.9)	19 (35.9)	33 (63.5)	33 (64.7)	**0.010**
CVA/TIA	10 (19.6)	10 (18.9)	11 (21.2)	12 (23.5)	0.940
Physiological measurements					
BMI (kg/m^2)	28.2 (24.2 – 31.2)	26.0 (22.1 – 30.8)	27.2 (22.6 – 33.8)	27.4 (22.7 – 34.1)	0.745
Pulse rate (bpm)	97 (80 – 113)	98 (77 – 109)	86 (71 – 110)	94 (74 – 110)	0.494
SBP (mmHg)	129 (110 – 150)	138 (118 – 160)	138 (124 – 152)	140 (122 – 152)	0.100
DBP (mmHg)	75 (64 – 86)	80 (61 – 96)	80 (67 – 93)	80 (72 – 90)	0.289

Variable	Q1 (n=51)	Q2 (n=53)	Q3 (n=52)	Q4 (n=51)	p-value†
Signs of fluid congestion					
Elevated JVP	36 (78.3)	36 (73.5)	35 (77.8)	36 (80)	0.890
Peripheral oedema	43 (84.3)	37 (69.8)	34 (65.4)	39 (76.5)	0.141
ECG rhythm					
SR	27 (52.9)	37 (69.8)	23 (44.2)	24 (47.1)	**0.040**
AF	22 (43.1)	14 (26.4)	26 (50)	25 (49)	0.052
Echocardiography measurements					
LVEDD (cm)	5.1 (4.6 – 5.9)	5.3 (4.8 – 6.1)	5.0 (4.5 – 5.5)	5.0 (4.5 - 5.7)	0.527
Dilated left ventricle	14 (36.8)	20 (44.4)	11 (25.6)	14 (32.6)	0.306
LVH	13 (34.2)	21 (47.7)	21 (48.8)	23 (53.5)	0.351
LVSD	24 (63.2)	34 (75.6)	24 (55.8)	29 (67.4)	0.265
Laboratory measurements (blood)					
BNP (pg/ml)	1164 (405 – 2049)	975 (382 – 2280)	622 (359 – 1247)	897 (392 – 2583)	0.105
Troponin I ≥ 0.04 (µg/L)*	30 (81.1)	30 (68.2)	21 (45.7)	19 (42.2)	**0.001**
Sodium (mmol/L)	136 (133 – 139)	137 (135 – 141)	139 (136 – 141)	139 (136 – 142)	**0.011**
Potassium (mmol/L)	4.1 (3.8 – 4.6)	4.2 (3.9 – 4.4)	4.0 (3.7 – 4.3)	4.1 (3.7 – 4.6)	0.320
Urea (mmol/L)	9.9 (6.9 – 14.2)	8.5 (5.6 – 12.2)	7.6 (5.9 – 9.7)	7.5 (5.9 – 10.0)	0.055
Creatinine (µmol/L)	103 (87 – 133)	115 (85 – 144)	93 (72 – 115)	101 (84 – 132)	**0.023**
eGFR (ml/min/1.73m^2)	55 (42 – 60)	52 (37 – 60)	60 (47 – 60)	58 (40 – 60)	0.160
eGFR <60ml/min/1.73m^2	30 (58.8)	32 (60.4)	20 (38.5)	29 (56.9)	0.088

Variable	Q1 (n=51)	Q2 (n=53)	Q3 (n=52)	Q4 (n=51)	p-value†
Cholesterol (total) (mmol/L)	3.9 (3.0 – 4.8)	3.9 (3.4 – 4.9)	4.9 (3.8 – 5.3)	3.8 (3.3 – 4.7)	**0.024**
HDL (mmol/L)	0.9 (0.7 – 1.3)	1.1 (0.9 – 1.40)	1.1 (1.0 – 1.5)	1.0 (0.8 – 1.2)	0.077
CRP (mg/L)	23 (10 – 51)	12 (5 – 41)	15 (6 – 28)	9 (6 – 17)	**0.004**
Cortisol (nmol/L)	328.4 (269.7 – 441.3)	367.1 (258.1 – 451.3)	336.3 (222.2 – 437.8)	362.7 (219.1 – 463.1)	0.852
11-deoxycortisol (pmol/L)	420.4 (190.0 – 765.0)	479.9 (314.4 – 1026.3)	634.8 (327.0 – 1124.0)	541.8 (366.0 – 1218.0)	0.054
11-deoxycortisol/cortisol (10^{-3})	1.27 (0.81 – 2.36)	1.57 (0.88 – 2.75)	1.88 (1.25 – 3.55)	1.88 (1.20 – 3.41)	**0.027**
Aldosterone (pmol/L)	41.1 (20.9 – 110.6)	81.2 (44.0 – 150.0)	80.6 (42.4 – 209.1)	147.3 (72.6 – 256.9)	**<0.001**
PRC (mIU/L)	121.4 (41.8 – 269.8)	46.0 (19.7 – 71.3)	16.1 (8.5 – 47.0)	8.1 (5.0 – 15.7)	**<0.001**
TSH (mIU/L)	1.7 (0.8 – 3.0)	1.9 (1.2 – 3.1)	1.8 (1.2 – 2.6)	1.7 (1.1 – 2.7)	0.888
Haemoglobin (g/dl)	12.9 (10.6 – 14.5)	12.6 (11.2 – 14.1)	12.5 (11.5 – 13.5)	12.0 (10.7 – 13.9)	0.616
Cardiovascular medication prior to admission					
Diuretic	33 (64.7)	24 (45.3)	31 (59.6)	24 (47.1)	0.131
Beta-blocker	22 (43.1)	16 (30.2)	20 (38.5)	20 (39.2)	0.577
Digoxin	11 (21.6)	3 (5.7)	7 (13.5)	8 (15.7)	0.132
Anti-arrhythmic	2 (3.9)	3 (5.7)	1 (1.9)	4 (7.8)	0.545
Aspirin	24 (47.1)	30 (56.6)	25 (48.1)	24 (47.1)	0.717
Statin	25 (49)	26 (49.1)	21 (40.4)	28 (54.9)	0.528

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).
† Kruskal-Wallis test was used for continuous variables and χ^2 test for categorical variables.
¥ Chi-Square approximation probably invalid
*measured at WIG and GRI

6.4 Discussion

6.4.1 Levels of RAAS mediators during hospital admission

In the current study, almost all patients had normal plasma aldosterone levels and approximately two thirds of patients had PRC within the normal range. That both aldosterone and PRC were not on average elevated appears somewhat paradoxical at first sight, given the fact that patients were admitted with decompensated HF and received treatment with diuretics early during hospital admission.

Traditionally, it has been accepted that worsening HF is characterised by RAAS activation. Nevertheless, most of the data about RAAS activation in decompensated HF come from studies in patients taking some form of a RAAS inhibitor with a diuretic (55) (339) (340). In the CONSENSUS study both renin and aldosterone levels were markedly elevated in patients with severe congestive HF not treated with an ACE inhibitor or ARB (55). Almost all patients were taking a diuretic in a high dose (mean dose of furosemide was 210mg) and over forty percent were treated with a high dose of aldosterone blocker (mean dose 80mg) (356), which are likely to account for the high RAAS activity reported in this study. CONSENSUS was a landmark trial, demonstrating the benefit of ACE inhibitors in HF and led to a wide acceptance of the concept of RAAS activation in patients with congestive HF. On the other hand, the available data about renin and aldosterone secretion in untreated patients with decompensated HF are sparse. Early studies five decades ago suggested that aldosterone secretion in patients with advanced congestive HF receiving treatment only with digitalis was not consistently increased (53). Similarly, patients with untreated congestive HF have been shown to have normal or low PRA (58) (357). The same pattern of findings was replicated in further studies of untreated patients with moderate and severe HF, which showed that not all patients had raised PRA or aldosterone (57) (358). Overall, the common finding in these studies with untreated HF patients was the absence of a universal RAAS activation. In

contrast, a considerable proportion of patients had normal or suppressed RAAS activity. The findings of my study are in accordance with these reports raising the question "have we been misled about the undisputable concept of RAAS activation in patients with worsening HF?"

The variation of RAAS activity in patients with HF has been attributed to the severity and state of cardio-circulatory decompensation as well as the extracellular fluid status (57). Organ and specifically renal perfusion is primarily involved in the regulation of RAAS. The MAP and extracellular volume determine renal perfusion and RAAS activation. Decline in cardiac output and in blood pressure in patients with decompensated HF lead to renal hypoperfusion and RAAS activation. Indeed, PRC was inversely related to SBP and DBP and patients with higher PRC were more likely to have LVSD and lower blood pressure in the current cohort (section 6.4.2). On the other hand, the activation of RAAS and other compensatory mechanisms lead to an expansion of the extracellular volume and an increase in MAP, which in turn exerts a negative feedback on renin and aldosterone secretion. Renin levels were lower in patients with decompensated HF with fluid overload compared with patients with decompensated HF without fluid congestion in a previous study (59). Moreover, low arterial blood pressure was a major stimulus for renin secretion in these patients. In the current study, the majority of patients had normal or high blood pressure and approximately three quarters had signs of fluid overload. Extracellular fluid overload increases the myocardial wall stress, which in turn stimulates the secretion of natriuretic peptides by the myocardium. Natriuretic peptides belong to the counter-regulatory pathways that are activated in HF and promote diuresis, vasodilatation and RAAS suppression (30) (124) (127). The raised natriuretic peptide levels during hospital admission along with the normal or high blood pressure are likely to account for the absence of gross RAAS activation in my cohort. In agreement with that view is also the finding that levels of RAAS mediators found in the current HF cohort

were similar to those reported in the healthy subjects following a high salt diet (section 3.3.3).

In summary, RAAS activity during hospital admission in patients not taking a RAAS inhibitor was not raised and the interplay between the haemodynamic status and the extracellular fluid volume and natriuretic peptides contributes to the variation of RAAS activity in these patients.

6.4.2 Patient characteristics according to RAAS activity during hospital admission

There were several significant differences present in patient characteristics according to the levels of RAAS mediators in this study. Aldosterone levels were higher in patients with higher PRC. Renin represents a surrogate of angiotensin II, which is one of the principal regulators of aldosterone synthesis in the adrenal glands. In addition, a trend for higher cortisol levels was present in patients with higher aldosterone levels; however, these differences were not statistically significant, indicating that the ACTH, which primarily regulates glucocorticoid secretion, is not a principal regulator of aldosterone biosynthesis in patients with decompensated HF. Moreover, the levels of serum potassium, which represents another major secretagogue for aldosterone, were not different among patients with different aldosterone levels. Overall, these findings suggest that aldosterone secretion in patients with decompensated HF is primarily regulated by the renin angiotensin system.

PRC was higher in patients with lower SBP and DBP. This association represents one of the fundamental responses involved in the homeostasis of the cardiovascular system. Lower blood pressure, as mentioned previously, leads to RAAS activation in order to preserve the systemic arterial pressure and organ perfusion. Moreover, patients with higher PRC represent a group characterised by more severe HF in my study. A trend for greater LV dilatation and

systolic dysfunction was present in patients with higher PRC, in keeping with previous studies (353) (354). In addition, patients with higher PRC were more likely to have elevated troponin, reflecting the severity of HF due to myocardial injury. In summary, higher PRC in patients with decompensated HF not taking a RAAS inhibitor was associated with myocardial necrosis and trended towards an association with LV remodeling, which both contribute to a decline in the cardiac output and potentially a decrease in blood pressure.

The decline in systemic perfusion due to low arterial pressure results in decreased renal blood flow, which has been reported as one of the strongest predictors of renal function in patients with HF (359). In the current study higher urea levels and a trend for higher creatinine levels were seen in patents with higher PRC. Traditionally, RAAS activation is considered to cause vasoconstriction to the glomerular arterioles in the context of a homeostatic mechanism to preserve filtration pressure and glomerular function in conditions characterised by low systemic and renal perfusion. That mechanism depends on different levels of constriction mediated by angiotensin II on the afferent and efferent arterioles and can potentially lead to lower filtration fraction and deterioration of kidney function in states of excessive vasoconstriction due to higher RAAS activity in patients with HF (360). In the current study, creatinine was higher in patients with higher PRC even in the subgroup with lower SBP, and that might indicate a negative influence of RAAS on renal function. However, no differences were seen in eGFR according to PRC and SBP levels. Moreover, the median SBP was well above 110mmHg in patients with lower SBP indicating that these patients were on average normotensive. Thus, no conclusions can be made about the association between PRC and renal function in relation to haemodynamic status in patients with decompensated HF in the current analyses.

A trend for lower sodium levels in patients with higher PRC was evident in patients with decompensated HF. Renin is synthesised in the JGA in kidneys and released in response to low sodium concentration at the macula densa, activation of the SNS and decrease of the intravascular volume (section 1.2.3). The differences in sodium levels according to PRC quartiles suggest that the above mechanism still operates in patients with decompensated HF in a pattern similar to that of a normal population. On the other hand, the RAAS activation due to low sodium in the macula densa reduces through angiotensin II the medullary blood flow and in turn increases the water reabsorption (361). Angiotensin II also stimulates the release of antidiuretic hormone and the thirst centre, resulting in further water reabsorption and an increase in water intake respectively (53). Thus, hyponatraemia in these patients, apart from a marker of hyper-reninaemia, represents a status of water excess in relation to extracellular sodium, indicating that the activation of water-retaining pathways becomes a predominant pathophysiological feature in patients with low serum sodium levels.
RAAS activation was also associated with higher CRP during hospital admission in patients not taking a RAAS inhibitor. Plasma CRP reflects the systemic inflammatory response with up-regulation of cytokines production in patients with HF; previous studies demonstrated that pro-inflammatory cytokines are activated in response to hypoperfusion and hypoxia in these patients (362). In addition, CRP has also been associated with the haemodynamic and neurohumoral responses related to the LV remodeling (363) (364). Thus, lower blood pressure and organ hypoperfusion are associated with RAAS activation and up-regulation of cytokines production and that might account for the higher CRP levels in patients with higher PRC.

Patients with lower PRC were more likely to have AF, a finding which appears somewhat unexpected in the first instance. AF is characterised by the loss of the atrial contraction, which itself contributes to decreased LV filling. The above effect potentially results in

reduction of the cardiac output and blood pressure with further reduction in renal perfusion and increase in RAAS activity. However, in this study, patients in AF were less likely to have LVSD compared with patients not in AF. Thus, patients with AF on admission represent a group with less severe HF, which potentially compensates for the aforementioned pathophysiological considerations and might partially explain why patients with lower PRC were more likely to have AF.

The lack of association between BNP and PRC in the current study is in keeping with previous findings in patients with LVSD treated with diuretics (365). Natriuretic peptides inhibit RAAS resulting in an inverse relationship with renin in normal volunteers (128). This reciprocal relationship is abrogated in patients with LVSD treated with diuretics which decrease the extravascular volume and in parallel stimulate RAAS activity (365). However, in patients with advanced HF, a positive correlation between natriuretic peptides and renin has been reported in some but not in all studies (353) (366). Patients with worse HF require higher doses of diuretics which by activating RAAS might contribute to the positive correlation between BNP and renin levels. The lack of positive association between PRC and BNP in my study, along with the finding of normal on average PRC levels despite diuretic treatment during hospital admission, may indicate that renin levels were suppressed by the raised natriuretic peptides in these patients. Alternatively, lower doses of diuretics might have been used compared with previous studies, as most of my patients were in NYHA class III during hospital admission, resulting into a lower degree of RAAS activation and lack of association between PRC and BNP levels.

Finally, the differences in baseline characteristics according to aldosterone to PRC ratio followed a similar pattern to that observed for PRC in an inverse fashion, indicating that the aldosterone to renin ratio is principally driven by renin in patients with HF. Interestingly, the

11-deoxycortisol to cortisol ratio was lower in patients with higher PRC and lower aldosterone to PRC ratio. To the best of my knowledge, this is a novel finding and is discussed further in chapter 8.

7. Chapter Seven - PRC and aldosterone levels at follow-up in patients not taking a RAAS blocker

7.1 Introduction

Diuretic therapy has been firmly established as one of the initial treatment strategies in patients with HF and fluid congestion during hospitalisation. Initiation of diuretics in patients with HF is associated with activation of RAAS (358) (367). In parallel, a decrease in natriuretic peptide levels due to extravascular volume reduction is evident in these patients. It is less clear if the discordance between the RAAS and natriuretic peptides seen following diuretic therapy persists in the medium- to long-term. That may be of importance as RAAS mediators exert detrimental effects on the cardiovascular system. Moreover, if the disconnection between BNP and RAAS activity continues over time, it indicates that apart from RAAS inhibition, augmentation of natriuretic peptide actions might be of therapeutic benefit in patients with chronic HF. Natriuretic peptides exert inhibitory effects on RAAS and SNS activity as well as natriuretic and vasodilating actions (30) (124) (127).

The main aim of this chapter is to describe the change in RAAS activity in relation to natriuretic peptide levels between hospital admission and the follow-up visit in patients not taking a RAAS inhibitor at both time points. Prior to this, I describe RAAS activity in patients not taking a RAAS inhibitor at follow-up. I also present the clinical characteristics according to RAAS mediators in these patients at the follow-up visit and examine if the associations seen between RAAS components and markers of HF severity during hospital admission continue to exist after discharge.

7.2 Methods

7.2.1 Study participants and laboratory measurements

Details of the study participants and the laboratory measurements were presented previously in sections 2.4.1 & 2.4.2. Only patients not receiving a RAAS inhibitor (ACE inhibitor/ARB or aldosterone blocker) at follow-up were included in the current study. For the comparisons of RAAS mediators between hospital admission and follow-up visit, the subgroup of patients not receiving a RAAS inhibitor prior to admission and at follow-up was included in the analyses.

7.2.2 Statistical analysis

All baseline characteristics are expressed as median (IQR) for continuous and absolute number (percentage) for categorical variables. The inter-group comparisons were carried out by the Mann-Whitney and Kruskal-Wallis test for continuous variables as appropriate and by the χ^2 test for categorical variables. For the comparisons of baseline characteristics between hospitalised and post-discharge patients not receiving a RAAS inhibitor, Wilcoxon matched pairs test and McNemar test were employed for continuous and categorical variables respectively. A p-value <0.05 was considered significant for all analyses. Statistical analyses were performed with Minitab version 15.

7.3 Results

7.3.1 Patient characteristics at follow-up stratified by treatment with a RAAS inhibitor

Of the 453 patients who completed the follow-up, 79 were not treated with a RAAS inhibitor after discharge (Table 7-1).

Patients not taking a RAAS inhibitor were older, more often women and more likely to have higher SBP and LVEF and less likely to have a history of previous angina and diabetes compared with patients taking a RAAS inhibitor or patients of the overall post-discharge cohort. Potassium and eGFR were lower in the former group compared with the other two groups. Patients not taking RAAS inhibitor were also less likely to be treated with a beta-blocker at the follow-up visit compared with patients of the other groups.

Table 7-1. Patient characteristics at the follow-up visit stratified by treatment with a RAAS inhibitor*

Variable	Patients not taking a RAAS inhibitor (n=79)	Patients taking a RAAS inhibitor (n=374)	Overall cohort – follow-up (n=453)	p-value¶	p-value†
Age (years)	75 (68 - 82)	71 (65 - 77)	72 (66 - 78)	**0.005**	**0.018**
Female gender	44 (55.7)	137 (36.6)	181 (40)	**0.002**	**0.009**
NYHA class					
I	3 (3.8)	9 (2.4)	12 (2.6)	0.484	0.569
II	52 (34.2)	236 (63.1)	288 (63.6)	0.648	0.701
III	24 (30.4)	125 (33.4)	149 (32.9)	0.601	0.660
IV	0 (0)	4 (1.1)	4 (0.9)	- ¥	- ¥
Medical history					
HF	29 (36.7)	159 (42.5)	188 (41.5)	0.341	0.424
MI	32 (40.5)	163 (43.6)	195 (43)	0.616	0.674
Angina	34 (43)	214 (57.2)	248 (54.8)	**0.021**	0.054
Diabetes mellitus	16 (20.3)	127 (34)	143 (31.6)	**0.017**	**0.043**
Hypertension	59 (74.7)	237 (63.4)	296 (65.3)	0.055	0.104
AF	46 (58.2)	194 (51.9)	240 (53)	0.304	0.388
CVA/TIA	15 (19)	76 (20.3)	91 (20.1)	0.788	0.821
Physiological measurements					
BMI (kg/m^2)	26.4 (23.5 - 32.3)	27.7 (23.9 - 32.7)	27.6 (23.8 - 32.6)	0.368	0.450
Weight (kg)	69.7 (57 - 85)	75.8 (64 - 90)	75 (62 - 89)	**0.027**	0.064

Variable	Patients not taking a RAAS inhibitor (n=79)	Patients taking a RAAS inhibitor (n=374)	Overall cohort – follow-up (n=453)	p-value¶	p-value†
Pulse rate (bpm)	80 (67 - 91)	73 (65 - 85)	74 (65 - 86)	0.090	0.155
SBP (mmHg)	138 (123 - 150)	126.5 (112 - 142)	129 (114 - 144)	**<0.001**	**0.002**
DBP (mmHg)	70 (59 - 79)	66 (58 - 76)	67 (58 - 76)	0.147	0.224
Signs of fluid congestion					
Elevated JVP	8 (11.3)	52 (16.4)	60 (15.4)	0.283	0.364
Peripheral oedema	30 (38)	123 (32.9)	153 (33.8)	0.385	0.468
ECG rhythm					
SR	47 (59.5)	222 (59.4)	269 (59.4)	0.982	0.985
AF	28 (35.4)	137 (36.6)	165 (36.4)	0.842	0.867
Echocardiogram measurements					
LVEF (%)	45 (34.5 - 53.5)	40 (30 - 46)	40 (31 - 48)	**<0.001**	**0.010**
LVEF >45%	38 (48.7)	100 (27.5)	138 (31.3)	**<0.001**	**0.003**
Laboratory measurements (blood)					
BNP (pg/ml)	471 (228 - 800)	387 (201 - 817)	396 (206 - 813)	0.367	0.450
Troponin I ≥ 0.04 (µg/L)	16 (20.3)	66 (17.7)	82 (18.1)	0.585	0.649
Sodium (mmol/L)	139 (138 - 141)	139 (137 - 141)	139 (137 - 141)	0.643	0.698
Potassium (mmol/L)	3.9 (3.6 - 4.1)	4.1 (3.8 - 4.4)	4.1 (3.8 - 4.3)	**0.001**	**0.006**
Urea (mmol/L)	9.4 (6.8 - 12)	8.5 (6.4 - 11.9)	8.6 (6.5 - 11.9)	0.600	0.660
Creatinine (µmol/L)	112 (89 - 149)	104.5 (87 - 128)	106 (97 - 130.5)	0.134	0.209

Variable	Patients not taking a RAAS inhibitor (n=79)	Patients taking a RAAS inhibitor (n=374)	Overall cohort – follow-up (n=453)	p-value¶	p-value†
eGFR (ml/min/1.73m^2)	51 (34 - 60)	60 (44 - 60)	59 (43 - 60)	**0.003**	**0.013**
eGFR <60ml/min/1.73m^2	49 (62)	181 (48.4)	47 (61.8)	**0.028**	0.065
Cholesterol (total) (mmol/L)	4.0 (3.6 - 5.4)	4.0 (3.3 - 4.8)	4.0 (3.3 - 4.9)	0.168	0.246
HDL (mmol/L)	1.2 (1.0 - 1.4)	1.0 (0.8 - 1.3)	1.1 (0.8 - 1.3)	**0.003**	**0.013**
CRP (mg/L)	6.2 (3.4 - 19.5)	5.1 (2.5 - 11.0)	5.2 (2.6 - 12)	**0.050**	0.103
TSH (mIU/L)	1.6 (0.9 - 3.0)	1.5 (0.9 - 2.3)	1.5 (0.9 - 2.4)	0.416	0.494
Haemoglobin (g/dl)	12.0 (11.1 - 13.4)	12.6 (11.2 - 13.7)	12.5 (11.2 - 13.6)	0.189	0.271
Cardiovascular medication					
Diuretic	78 (98.7)	367 (98.1)	445 (98.2)	0.710	0.750
Beta-blocker	45 (57)	264 (70.6)	309 (68.2)	**0.018**	0.051
Digoxin	17 (21.5)	98 (26.2)	115 (25.4)	0.385	0.463
Anti-arrhythmic	7 (8.9)	19 (5.1)	26 (5.7)	0.189	0.289
Aspirin	46 (58.2)	207 (55.4)	253 (55.9)	0.639	0.694
Statin	56 (70.9)	279 (74.6)	335 (74)	0.494	0.569

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).
* ACE inhibitor/ARB or aldosterone blocker
¶ Patients not taking a RAAS inhibitor (n=76) vs patients taking a RAAS inhibitor (n=377), Mann-Whitney test for continuous and χ^2 test for categorical variables.
† Patients not taking a RAAS inhibitor (n=76) vs patients of the overall post-discharge cohort (n=453), Mann-Whitney test for continuous and χ^2 test for categorical variables
¥ Chi-Square approximation probably invalid

7.3.2 RAAS activity during follow-up visit

Levels of aldosterone, PRC and aldosterone to PRC ratio during follow-up are presented below.

7.3.2.1 Aldosterone

An aldosterone level measured during follow-up was available in 77 of the 79 patients. The median (IQR) aldosterone was 182.4 (92.6 - 329) pmol/L (Figure 7-1) and the mean (SD) aldosterone was 273.1 (363.7) pmol/L. A frequency distribution histogram of aldosterone levels in these patients is displayed in Figure 7-2. The distribution of aldosterone concentrations was positively skewed. The minimum aldosterone value was 21.1 pmol/L and the maximum aldosterone value was 2314.1 pmol/L. Almost all patients (97.5%) had aldosterone levels within the normal range (0 - 937 pmol/L).

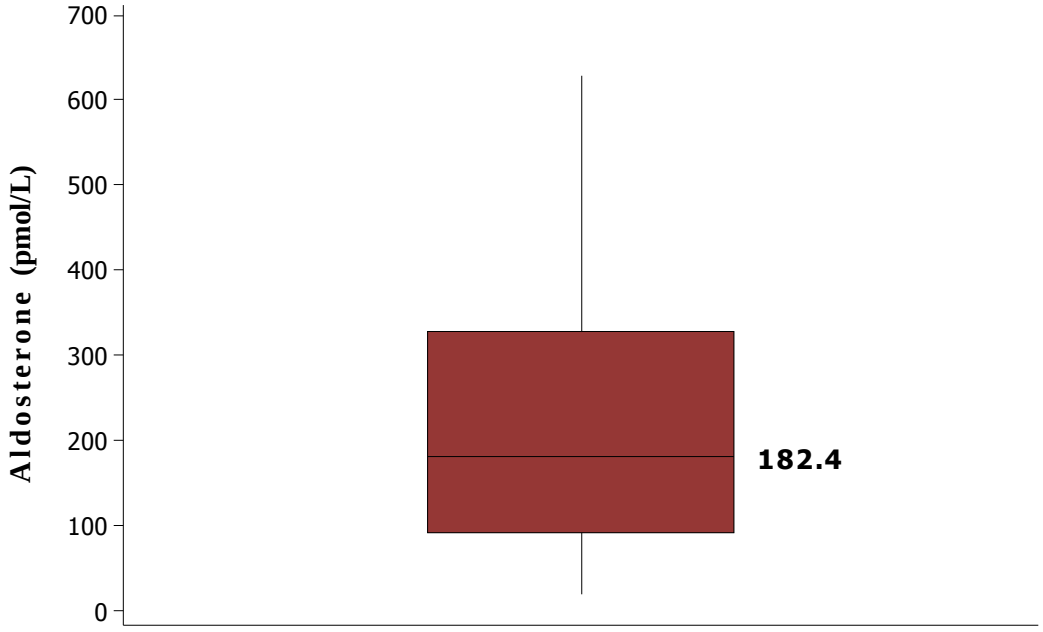


Figure 7-1. Box and whisker plot of aldosterone levels showing the 2.5, 25, 50, 75 and 97.5 centiles

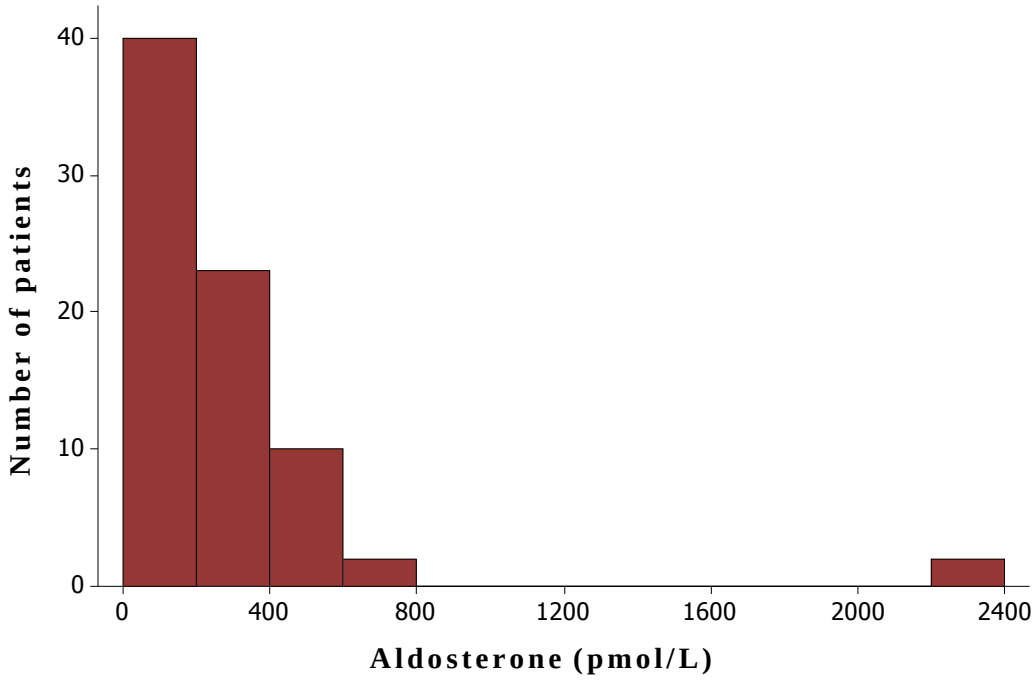


Figure 7-2. Frequency distribution histogram of aldosterone concentrations in patients not receiving an ACE inhibitor/ARB or aldosterone blocker

7.3.2.2 PRC

A PRC measured during follow-up was available in 78 of the 79 patients. The median (IQR) PRC was 47.5 (18.9 - 107.1) mIU/L (Figure 7-3) and the mean (SD) PRC was 77.6 (86.7) mIU/L. A frequency distribution histogram of PRC levels in these patients is displayed in Figure 7-4. The distribution of PRC values was positively skewed. The minimum PRC was 5.0 mIU/L and the maximum PRC was 423.6 mIU/L. Approximately half of patients (48%) had PRC within the normal range (5.0 - 44.9 mIU/L).

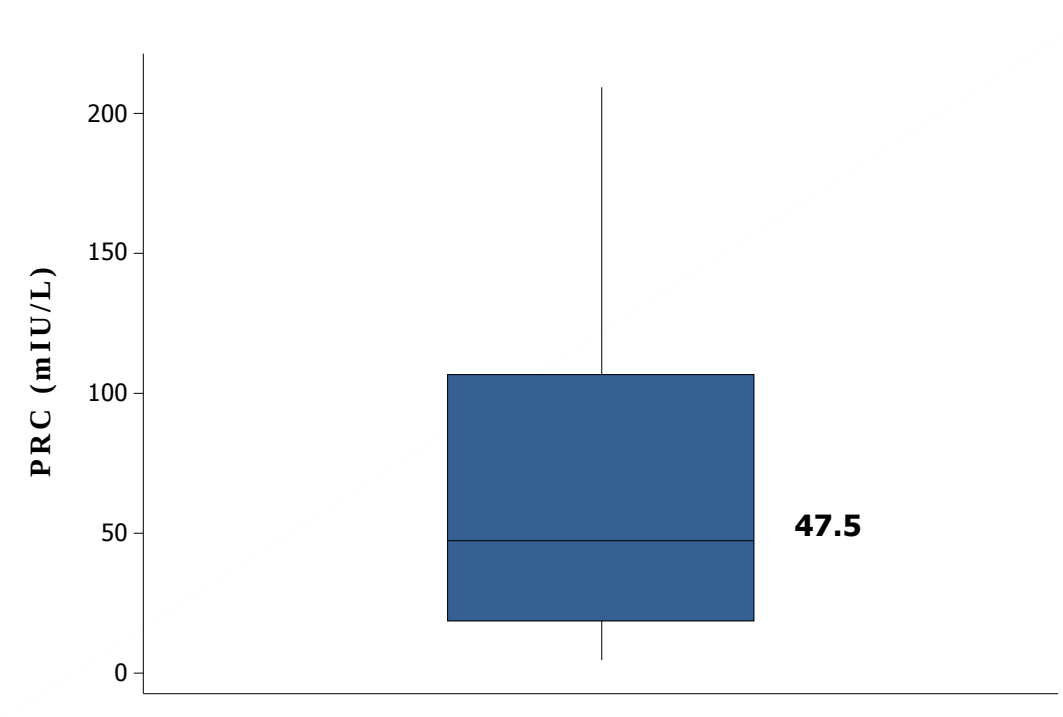


Figure 7-3. Box and whisker plot of PRC levels showing the 2.5, 25, 50, 75 and 97.5 centiles

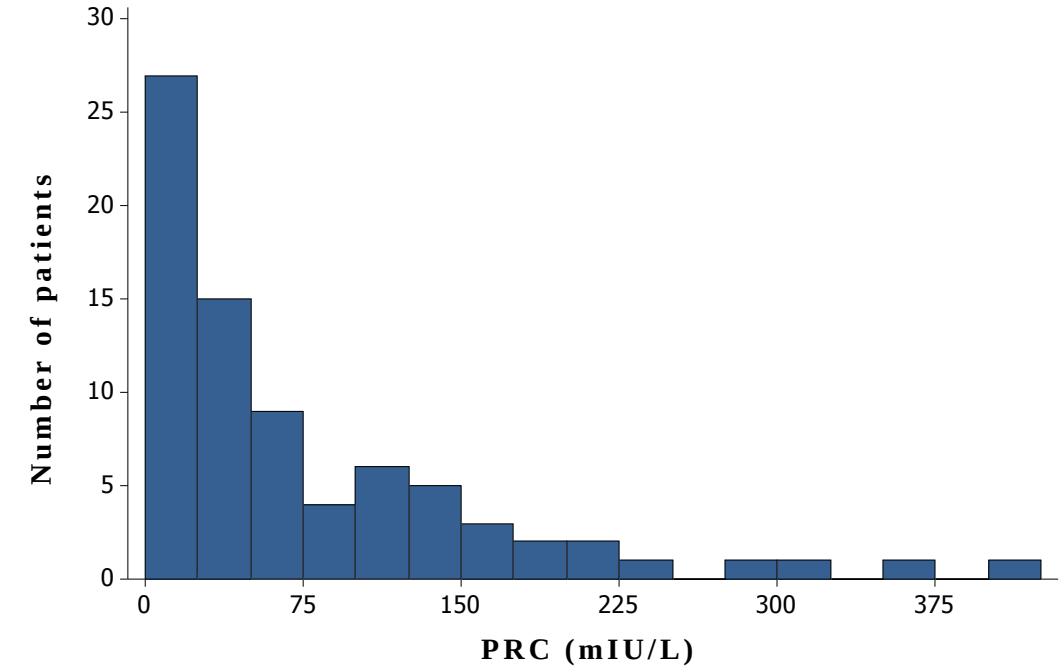


Figure 7-4. Frequency distribution histogram of PRC in patients not receiving an ACE inhibitor/ARB or aldosterone blocker

7.3.2.3 Aldosterone to PRC

An aldosterone to PRC ratio could be calculated for 76 of the 79 patients. The median (IQR) aldosterone to PRC was 3.74 (1.88 - 9.73) (Figure 7-5) and the mean (SD) aldosterone to PRC was 7.2 (8.2). A frequency distribution histogram of aldosterone to PRC levels in these patients is displayed in Figure 7-6. The distribution of aldosterone to PRC values was positively skewed. The minimum aldosterone to PRC was 0.33 and the maximum aldosterone to PRC was 36.5.

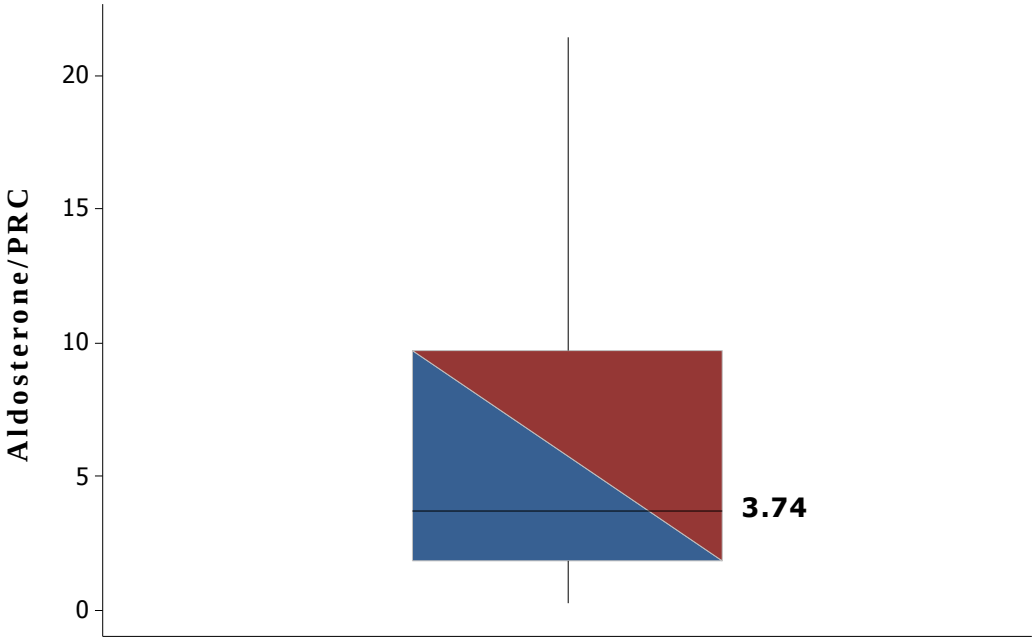


Figure 7-5. Box and whisker plot of aldosterone to PRC values showing the 2.5, 25, 50, 75 and 97.5 centiles

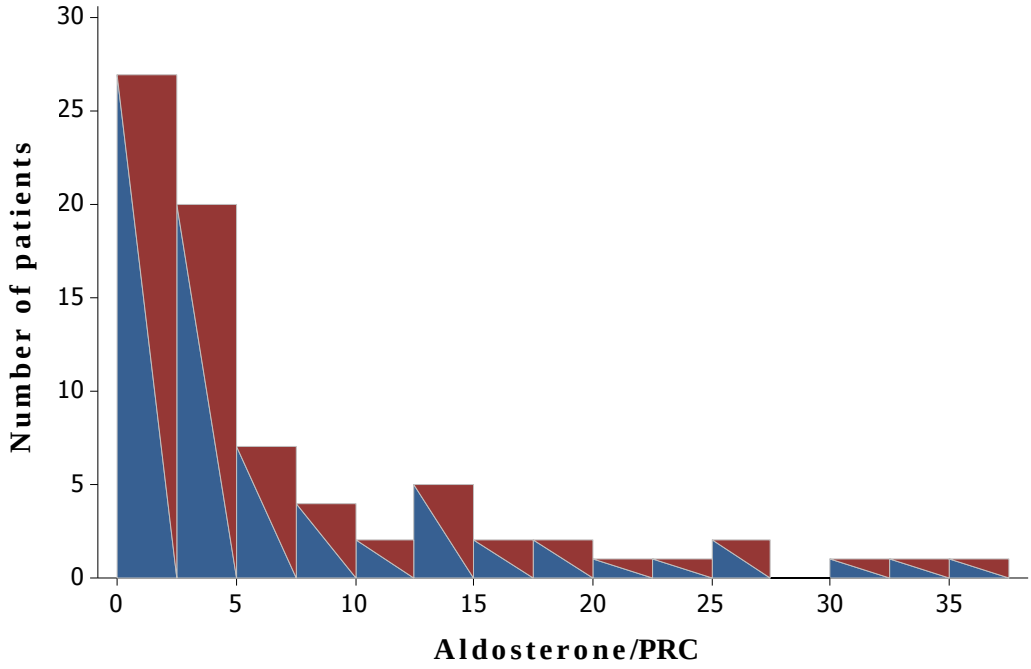


Figure 7-6. Frequency histogram of aldosterone to PRC in patients not receiving an ACE inhibitor/ARB or aldosterone blocker

7.3.3 Patient characteristics according to RAAS activity

The characteristics of patients not receiving a RAAS inhibitor during follow-up were stratified according to the levels of aldosterone and PRC and the aldosterone to PRC ratio.

7.3.3.1 Patient characteristics according to aldosterone levels

The cohort of 77 patients with measured aldosterone levels was divided into two groups according to the median aldosterone; patients with aldosterone levels <182.4 pmol/L and patients with aldosterone levels ≥ 182.4 pmol/L (Table 7-2).

Patients with higher aldosterone levels were more likely to have higher PRC and cortisol and higher aldosterone to PRC ratio. These patients also had higher cholesterol and lower TSH and were more likely to have a history of hypertension compared to patients with lower aldosterone levels.

Table 7-2. Characteristics of patients not taking an ACE inhibitor/ ARB or aldosterone blocker according to the median aldosterone

Variable	Aldosterone < 182.4 pmol/L (n=39)	aldosterone ≥ 182.4 pmol/L (n=38)	p-value†
Age (years)	77 (71 – 83)	73 (68 – 79)	0.081
Female gender	21 (53.8)	23 (60.5)	0.554
NYHA class			
I	1 (2.6)	2 (5.3)	0.541
II	27 (69.2)	25 (65.8)	0.747
III	11 (28.2)	11 (29)	0.943
Medical history			
HF	14 (35.9)	14 (36.8)	0.931
MI	14 (35.9)	16 (42.1)	0.577
Angina	13 (33.3)	20 (52.6)	0.087
Diabetes mellitus	6 (15.4)	9 (23.7)	0.358
Hypertension	25 (64.1)	32 (84.2)	**0.044**
AF	25 (64.1)	21 (55.2)	0.429
CVA/TIA	9 (23.1)	5 (13.2)	0.259
Physiological measurements			
BMI (kg/m^2)	25.1 (23.0 – 32.9)	26.8 (23.9 – 32.4)	0.575
Pulse rate (bpm)	73 (64 – 84)	84 (67 – 94)	0.179
SBP (mmHg)	138 (123 – 150)	141 (126 – 152)	0.658
DBP (mmHg)	69 (60 – 75)	71 (57 – 81)	0.695
Signs of fluid congestion			
Elevated JVP	4 (11.8)	3 (8.6)	0.660
Peripheral oedema	15 (38.5)	14 (36.8)	0.883
ECG rhythm			
SR	20 (51.3)	26 (68.4)	0.125
AF	17 (43.6)	10 (26.3)	0.112
Echocardiography measurements			
LVEF	46 (38 – 56)	45 (34 – 53)	0.720
LVEF <45%	18 (46.2)	17 (46)	0.985
Laboratory measurements (blood)			
BNP (pg/ml)	525 (260 – 800)	411 (196 – 758)	0.292
Troponin I ≥ 0.04 (µg/L)	7 (18)	8 (21.1)	0.731

Variable	Aldosterone < 182.4 pmol/L (n=39)	aldosterone ≥ 182.4 pmol/L (n=38)	p-value†
Sodium (mmol/L)	140 (138 – 141)	139 (138 – 140)	0.104
Potassium (mmol/L)	4.0 (3.6 – 4.3)	3.9 (3.5 – 4.1)	0.129
Urea (mmol/L)	9.3 (6.3 – 14.0)	9.8 (7.2 – 11.5)	0.992
Creatinine (µmol/L)	118 (91 – 142)	112 (84 – 157)	0.915
eGFR (ml/min/1.73m^2)	52 (39 – 60)	50 (32 – 60)	0.768
eGFR <60ml/min/1.73m^2	24 (61.5)	24 (63.2)	0.883
Cholesterol (total) (mmol/L)	3.8 (3.1 – 5.1)	4.6 (3.7 – 5.5)	**0.030**
HDL (mmol/L)	1.2 (0.9 – 1.3)	1.1 (1.0 – 1.5)	0.337
CRP (mg/L)	5.5 (3.2 – 19.3)	7.4 (4.3 – 23.0)	0.425
TSH (mIU/L)	1.7 (1.1 – 3.8)	1.3 (0.7 – 2.4)	**0.044**
Cortisol (nmol/L)	209.8 (149.0 – 288.7)	263.7 (172.3 – 343.7)	0.051
11-deoxycortisol (pmol/L)	522.8 (295.3 – 794.7)	451.7 (311.4 – 643.8)	0.573
11-deoxycortisol/cortisol (10^{-3})	2.67 (1.44 – 4.43)	1.69 (1.18 – 3.10)	0.163
PRC (mIU/L)	28.5 (11.8 – 50.7)	68.1 (31.1 – 159.7)	**<0.001**
Aldosterone/PRC	3.11 (1.35 – 7.08)	4.63 (2.22 – 13.18)	**0.042**
Haemoglobin (g/dl)	11.7 (11.0 – 13.1)	12.1 (11.4 – 13.4)	0.328
Cardiovascular medication			
Diuretic	38 (97.4)	38 (100)	- ¥
Beta-blocker	26 (66.7)	18 (47.4)	0.087
Digoxin	10 (25.6)	7 (18.4)	0.445
Anti-arrhythmic	2 (5.1)	5 (13.2)	0.220
Aspirin	22 (56.4)	22 (57.9)	0.895
Statin	29 (74.4)	27 (71.1)	0.745

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)

† Mann-Whitney test was used for continuous variables and χ^2 test for categorical variables.

¥ Chi-Square approximation probably invalid.

7.3.3.2 Patient characteristics according to PRC

The cohort of 78 patients with measured PRC was divided into two groups according to the median PRC; patients with PRC <47.5 and patients with PRC ≥ 47.5 (Table 7-3). Patients with higher PRC were more likely to have higher BMI, aldosterone and cortisol. These patients also had lower potassium and aldosterone to PRC ratio compared to patients with lower PRC. Beta-blockers were prescribed more often in patients with lower compared to patients with higher PRC.

Table 7-3. Characteristics of patients not taking an ACE inhibitor/ ARB or aldosterone blocker according to the median PRC

Variable	PRC < 47.5 (n=39)	PRC ≥ 47.5 (n=39)	p-value†
Age (years)	76 (69 – 83)	73 (67 – 79)	0.353
Female gender	23 (59)	20 (51.3)	0.495
NYHA class			
I	3 (7.7)	0 (0)	0.077
II	26 (66.7)	25 (64.1)	0.812
III	10 (25.6)	14 (35.9)	0.326
Medical history			
HF	11 (28.1)	18 (46.2)	0.101
MI	14 (35.9)	18 (46.2)	0.357
Angina	14 (35.9)	20 (51.3)	0.171
Diabetes mellitus	7 (18)	9 (23.1)	0.575
Hypertension	27 (69.2)	31 (79.5)	0.300
AF	22 (56.4)	23 (59)	0.819
CVA/TIA	8 (20.5)	7 (18)	0.774
Physiological measurements			
BMI (kg/m^2)	24.6 (22.9 – 28.7)	28.9 (25.0 – 33.5)	**0.024**
Pulse rate (bpm)	75 (63 – 90)	82 (67 – 93)	0.358
SBP (mmHg)	143 (124 – 156)	134 (121 – 145)	0.185
DBP (mmHg)	69 (64 – 82)	70 (58 – 76)	0.614
Signs of fluid congestion			
Elevated JVP	2 (5.7)	6 (17.1)	0.133
Peripheral oedema	11 (28.2)	18 (46.2)	0.101
ECG rhythm			
SR	24 (61.5)	23 (59)	0.817
AF	14 (35.9)	13 (33.3)	0.812
Echocardiography measurements			
LVEF	45 (35 – 57)	45.5 (33 – 50)	0.373
LVEF <45%	19 (48.7)	18 (47.4)	0.906
Laboratory measurements (blood)			
BNP (pg/ml)	505 (215 – 800)	457 (228 – 819)	0.772
Troponin I ≥ 0.04 (µg/L)	7 (18)	9 (23.1)	0.575
Sodium (mmol/L)	140 (138 – 141)	139 (137 – 140)	0.074
Potassium (mmol/L)	4.0 (3.8 - 4.2)	3.7 (3.4 – 4.1)	**0.029**
Urea (mmol/L)	9.4 (6.3 – 14.4)	9.8 (7.3 – 12.0)	0.857

Variable	PRC < 47.5 (n=39)	PRC ≥ 47.5 (n=39)	p-value†
Creatinine (µmol/L)	111 (82 – 134)	118 (92 – 155)	0.299
eGFR (ml/min/1.73m^2)	55 (35 – 60)	45 (33 – 60)	0.221
eGFR <60ml/min/1.73m^2	21 (53.9)	28 (71.8)	0.101
Cholesterol (total) (mmol/L)	3.9 (3.2 – 5.5)	4.1 (3.6 – 5.2)	0.697
HDL (mmol/L)	1.2 (1.0 – 1.3)	1.2 (0.9 – 1.5)	0.930
CRP (mg/L)	4.6 (3.1 – 17.8)	8.9 (4.6 – 19.5)	0.187
TSH (mIU/L)	1.6 (0.9 – 2.9)	1.6 (1.0 – 3.1)	0.883
Cortisol (nmol/L)	197.1 (130.0 – 294.2)	263.9 (191.0 – 328.6)	**0.032**
11-deoxycortisol (pmol/L)	531 (256 – 715)	452 (316 – 779)	0.982
11-deoxycortisol/cortisol (10^{-3})	2.46 (1.49 – 3.97)	1.74 (1.18 – 3.35)	0.165
Aldosterone (pmol/L)	112.9 (58.7 – 236.6)	250.5 (172.4 – 457.3)	**<0.001**
Aldosterone/PRC	6.72 (3.21 – 15.15)	2.63 (1.42 – 4.34)	**<0.001**
Haemoglobin (g/dl)	11.9 (11.1 – 13.1)	12.1 (11.4 – 13.7)	0.439
Cardiovascular medication			
Diuretic	38 (97.4)	39 (100)	- ¥
Beta-blocker	27 (69.2)	18 (46.2)	**0.039**
Digoxin	7 (18)	9 (23.1)	0.575
Anti-arrhythmic	2 (5.1)	5 (12.8)	0.235
Aspirin	26 (66.7)	20 (51.3)	0.167
Statin	29 (74.4)	26 (66.7)	0.456

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)

† Mann-Whitney test was used for continuous variables and χ^2 test for categorical variables.

¥ Chi-Square approximation probably invalid.

7.3.3.3 Patient characteristics according to aldosterone to PRC ratio

The cohort of 76 patients with calculated aldosterone to PRC ratio was divided into two groups according to the median aldosterone to PRC; patients with aldosterone/PRC <3.74 and patients with aldosterone/PRC ≥ 3.74 (Table 7-4)

Patients with lower aldosterone to PRC ratio were more likely to be in NYHA functional class III and less likely to be in NYHA functional class II compared to patients with higher aldosterone to PRC ratio. These patients were also more likely to have history of MI and angina and elevated troponin. PRC, BNP, urea and creatinine were higher in patients with lower aldosterone to PRC ratio compared with patients with higher aldosterone to PRC ratio. Conversely, aldosterone and eGFR were lower in the former compared with the latter group. Apart from the above differences, a trend for lower SBP and DBP and higher CRP was also present in patients with lower aldosterone to PRC ratio.

Table 7-4. Characteristics of patients not taking an ACE inhibitor/ARB or aldosterone blocker according to the median aldosterone to PRC ratio

Variable	Aldosterone to PRC < 3.74 (n=38)	Aldosterone to PRC ≥ 3.74 (n=38)	p-value†
Age (years)	76 (71 – 82)	75 (68 – 81)	0.366
Female gender	19 (50)	24 (63.2)	0.247
NYHA class			
I	0 (0)	3 (7.8)	0.077
II	21 (55.3)	30 (79)	**0.028**
III	17 (44.7)	5 (13.2)	**0.002**
Medical history			
HF	18 (47.4)	10 (26.3)	0.057
MI	20 (52.6)	10 (26.3)	**0.019**
Angina	21 (55.3)	12 (31.6)	**0.037**
Diabetes mellitus	8 (21.1)	7 (18.4)	0.773
Hypertension	27 (71.1)	29 (76.3)	0.602
AF	20 (52.6)	25 (65.8)	0.243
CVA/TIA	3 (7.9)	11 (29)	**0.018**
Physiological measurements			
BMI (kg/m^2)	27.1 (24.3 – 32.9)	26.1 (22.7 – 32.2)	0.406
Pulse rate (bpm)	74 (67 – 89)	80 (65 – 93)	0.585
SBP (mmHg)	133 (119 – 153)	143 (128 – 148)	0.314
DBP (mmHg)	69 (58 – 74)	73 (59 – 82)	0.273
Signs of fluid congestion			
Elevated JVP	4 (11.7)	3 (8.8)	0.690
Peripheral oedema	17 (44.7)	11 (29)	0.154
ECG rhythm			
SR	25 (65.8)	21 (55.3)	0.348
AF	10 (26.3)	16 (42.1)	0.147
Echocardiography measurements			
LVEF	45 (29 – 52)	46 (37 – 55)	0.346
LVEF <45%	8 (57.2)	27 (44.3)	0.384
Laboratory measurements (blood)			
BNP (pg/ml)	604 (296 – 1017)	386 (183 – 703)	**0.040**
Troponin I ≥ 0.04 (µg/L)	11 (29)	4 (10.5)	**0.044**
Sodium (mmol/L)	140 (138 – 141)	139 (137 – 141)	0.872
Potassium (mmol/L)	3.8 (3.5 – 4.1)	4.0 (3.7 – 4.2)	0.149

Variable	Aldosterone to PRC < 3.74 (n=38)	Aldosterone to PRC ≥ 3.74 (n=38)	p-value†
Urea (mmol/L)	10.3 (7.4 – 14.4)	7.9 (6.3 – 10.7)	**0.025**
Creatinine (µmol/L)	125 (96 – 155)	104 (82 – 129)	**0.020**
eGFR (ml/min/1.73m^2)	42 (33 – 60)	57 (42 – 60)	**0.047**
eGFR <60ml/min/1.73m^2	28 (73.7)	20 (52.6)	0.057
Cholesterol (total) (mmol/L)	3.9 (3.3 – 4.5)	4.7 (3.7 – 5.9)	**0.019**
HDL (mmol/L)	1.2 (0.8 – 1.5)	1.1 (1.0 – 1.3)	0.858
CRP (mg/L)	9.1 (4.6 – 20.5)	4.7 (2.4 – 15.0)	0.083
TSH (mIU/L)	1.6 (0.9 – 3.7)	1.6 (0.9 – 2.8)	0.553
Cortisol (nmol/L)	229.8 (167.7 – 308.0)	222.6 (137.4 – 313.8)	0.670
11-deoxycortisol (pmol/L)	468.7 (318.0 – 698.2)	447 (198.5 – 752.0)	0.374
11-deoxycortisol/cortisol (10^{-3})	2.02 (1.29 – 3.51)	2.13 (1.31 – 3.84)	0.973
Aldosterone (pmol/L)	135.1 (57.1 – 268.3)	241.9 (119.4 – 361.4)	**0.012**
PRC (mIU/L)	88.2 (32.9 – 159.7)	23.1 (10.4 – 53.2)	**<0.001**
Haemoglobin (g/dl)	11.7 (10.7 – 13.2)	12.2 (11.4 – 13.5)	0.102
Cardiovascular medication			
Diuretic	38 (100)	37 (97.4)	**- ¥**
Beta-blocker	21 (55.3)	23 (60.5)	0.642
Digoxin	8 (21.1)	8 (21.1)	1.000
Anti-arrhythmic	5 (13.2)	2 (5.3)	0.234
Aspirin	25 (65.8)	19 (50)	0.163
Statin	30 (79)	25 (65.8)	0.200

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)

† Mann-Whitney test was used for continuous variables and χ^2 test for categorical variables.

¥ Chi-Square approximation probably invalid.

7.3.4 RAAS activity during hospital admission and follow-up in patients not taking a RAAS inhibitor

Of the 79 patients not taking an ACE inhibitor/ARB or aldosterone blocker at follow-up, 57 patients were not taking the above agents prior to hospital admission. The demographic characteristics, medical history and LVEF of these patients are presented in Table 7-5.

Table 7-5. Demographics, medical history and echocardiography measurements in patients not taking a RAAS inhibitor* prior to admission and during follow-up (n=57) and in patients of the overall cohort during admission (n=722) and follow-up (n=453)

Variable	Patients not taking a RAAS inhibitor (n=57)	Overall cohort – hospital (n=722)	Overall cohort – follow-up (n=453)	p-value¶	p-value†
Age (years)	76 (69 - 82.5)	74 (68 - 81)	72 (66 - 78)	0.309	**0.009**
Female gender	32 (56.1)	332 (46)	181 (40)	0.139	**0.002**
Medical history					
HF	15 (26.3)	320 (44.3)	188 (41.5)	**0.008**	**0.027**
MI	21 (36.8)	322 (44.6)	195 (43)	0.256	0.372
Angina	27 (47.4)	396 (54.8)	248 (54.7)	0.275	0.292
Diabetes mellitus	9 (15.8)	227 (31.4)	143 (31.6)	**0.013**	**0.014**
Hypertension	41 (71.9)	478 (66.2)	296 (65.3)	0.378	0.322
AF	32 (56.1)	387 (53.6)	240 (53)	0.711	0.652
CVA/TIA	13 (22.8)	155 (21.5)	91 (20.1)	0.813	0.631
Echocardiography measurements					
LVEF (%)	45.5 (36.5 - 55)	-	40 (31 - 48)	-	**0.002**
LVSD (Y)	24 (54.6)	341 (66.6)	-	0.106	-

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)
*ACE inhibitor/ARB or aldosterone blocker
¶ Patients not taking a RAAS inhibitor prior to admission and during follow-up (n=57) vs patients of the overall hospitalised cohort (n=722), Mann-Whitney test for continuous and χ^2 test for categorical variables.
† Patients not taking a RAAS inhibitor prior to admission and during follow-up (n=57) vs patients of the overall post-discharge cohort (n=453), Mann-Whitney test for continuous and χ^2 test for categorical variables.

Patients not taking a RAAS inhibitor during hospital admission and at follow-up were older, more often women and more likely to have higher LVEF compared with patients of the overall post-discharge cohort. These patients were also less likely to have a history of HF and diabetes compared with patients of the overall post-discharge and hospitalised cohort.

The physiological and laboratory measurements of these patients and the medication during hospital admission and follow-up are presented in Table 7-6.

Table 7-6. Clinical characteristics, physiological and laboratory measurements and medication in patients not taking a RAAS inhibitor* during hospital admission and follow-up (n=57)

Variable	During admission (n=57)	At follow-up (n=57)	p-value†
NYHA class			
I	0 (0)	2 (3.5)	0.500
II	20 (35.1)	38 (66.7)	**0.001**
III	30 (52.6)	17 (29.8)	**0.026**
IV	7 (12.3)	0 (0)	**0.016**
Physiological measurements			
BMI (kg/m^2)	28 (24 - 34)	26.3 (23 - 33.3)	**<0.001**
Weight (kg)	73 (57 - 89.8)	69 (55.5 - 86)	**<0.001**
Pulse rate (bpm)	86 (72 - 99.5)	79 (67.5 - 92)	**0.013**
SBP (mmHg)	140 (125 - 155)	142 (126 - 152.5)	0.720
DBP (mmHg)	78 (65 - 89.5)	69 (58 - 79.5)	**0.002**
Pulse pressure (mmHg)	58 (50 - 137)	72 (28 - 90)	0.080
Signs of fluid congestion			
Elevated JVP	41 (79)	4 (8)	**<0.001**
Peripheral oedema	38 (66.7)	21 (36.8)	**<0.001**
ECG rhythm			
SR	37 (64.9)	37 (64.9)	1
AF	18 (31.6)	19 (33.3)	1
Laboratory measurements (blood)			
BNP (pg/ml)	647 (310 - 1792)	457 (211 - 784)	**<0.001**
Sodium (mmol/L)	138 (126 - 138)	139 (137.5 - 141)	**0.040**
Potassium (mmol/L)	4.2 (3.8 - 4.5)	3.9 (3.7 - 4.2)	**0.001**
Urea (mmol/L)	8.8 (5.7 - 10.9)	9.2 (6.8 - 11.8)	0.258
Creatinine (µmol/L)	112 (75 - 142.5)	115 (88 - 150)	**0.003**
eGFR (ml/min/1.73m^2)	53 (35 - 60)	51 (32 - 60)	0.061
eGFR <60ml/min/1.73m^2	35 (61.4)	37 (64.9)	0.727
Cholesterol (total) (mmol/L)	3.9 (3.3 - 5.1)	4.2 (3.6 - 5.5)	0.139
HDL (mmol/L)	1.1 (0.9 - 1.4)	1.15 (1.0 - 1.4)	0.261
CRP (mg/L)	11 (4.7 - 38)	6.3 (3.8 - 21)	**0.001**
TSH (mIU/L)	2.3 (1.1 - 3.7)	1.6 (0.7 - 2.6)	0.393
Cortisol (nmol/L)	358.2 (260.8 - 497.9)	222.9 (151.6 - 298.4)	**<0.001**
11-deoxycortisol (pmol/L)	536 (319 - 1068)	423.8 (257.9 - 772.8)	0.557
11-deoxycortisol/cortisol (10^{-3})	1.68 (1.12 - 3.4)	2.03 (1.29 - 3.44)	0.174

Variable	During admission (n=57)	At follow-up (n=57)	p-value†
Aldosterone (pmol/L)	113.8 (53.5 - 250.8)	181.5 (93.7 - 293.6)	0.132
PRC (mIU/L)	34.2 (9.1 - 67.6)	42.8 (17.9 - 104.2)	**0.036**
Aldosterone/PRC	3.2 (1.7 - 8.1)	3.9 (2.1 - 12.6)	0.307
Haemoglobin (g/dl)	12 (10.3 - 13.3)	12 (10.9 - 13.4)	0.380
Cardiovascular medication¶			
Diuretic	30 (52.6)	56 (98.2)	**<0.001**
Beta-blocker	28 (49.1)	29 (50.9)	1
Digoxin	8 (14)	9 (15.8)	1
Anti-arrhythmic	5 (8.8)	5 (8.8)	1
Aspirin	32 (56.1)	33 (57.9)	1
Statin	34 (59.6)	39 (68.4)	0.267

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)

*ACE inhibitor/ARB or aldosterone blocker.

† Wilcoxon matched pairs test was used for continuous variables and McNemars's test was used for categorical variables.

¶ medication prior to hospital admission for patients studied during admission.

7.3.4.1 Baseline characteristics stratified by aldosterone to PRC median

PRC levels were higher at follow-up compared with hospital admission. There was a trend for higher aldosterone levels and higher aldosterone to PRC ratio after discharge but that failed to reach statistical significance. Higher proportion of patients was treated with a diuretic after discharge compared with hospital admission. Moreover, the BNP and weight were lower at follow-up. In addition, the majority of patients were in NYHA class III during hospital admission and in NYHA class II at the follow-up visit. Finally, the SBP, pulse rate, CRP, cortisol and potassium were also lower and creatinine and sodium levels were higher after discharge compared with hospital admission. The disconnection between BNP levels and RAAS activity in these patients between hospital admission and the follow-up visit is presented in Figure 7-7 & Figure 7-8.

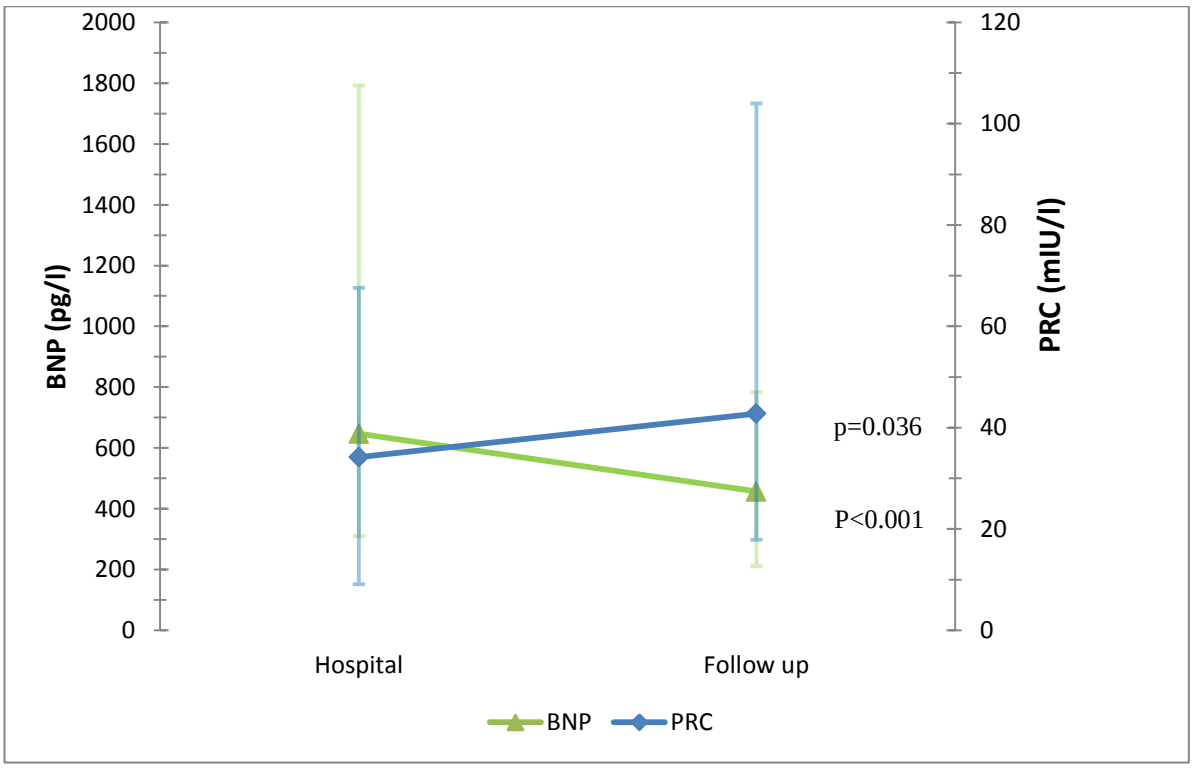


Figure 7-7. BNP and PRC levels during hospital admission and at follow-up

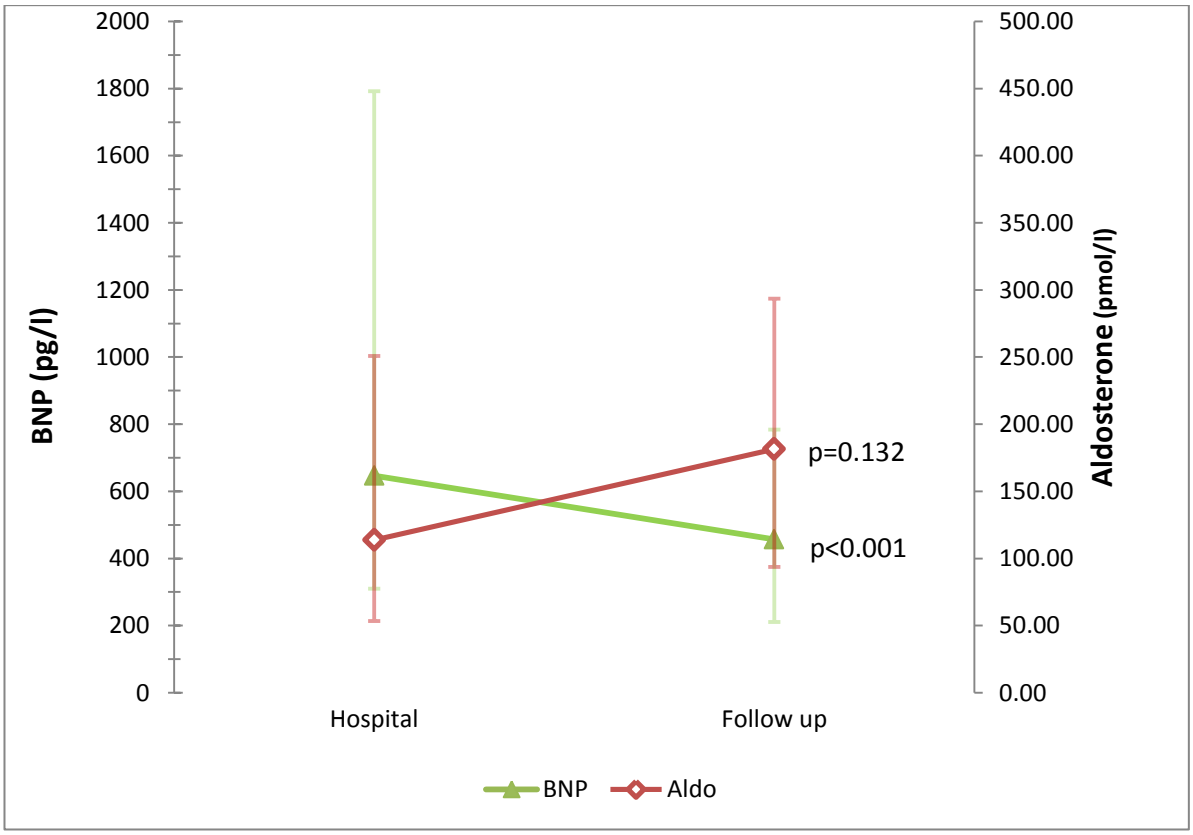


Figure 7-8. BNP and aldosterone levels during hospital admission and at follow-up

7.4 Discussion

7.4.1 Patients characteristics according to RAAS activity during follow-up

Patients studied in this chapter were not taking a RAAS inhibitor at the follow-up visit. These patients were older and more often female compared with patients taking these agents at follow-up. These characteristics are more often seen in patients with HFpSF. Indeed, this group had higher LVEF at the follow-up visit with a higher proportion of patients having LVEF>45% compared with the group of patients taking a RAAS inhibitor. Although RAAS inhibitors have been one of the cornerstone treatments in patients with HFrSF, no definitive prognostic benefit has been shown with these agents in patients with HFpSF (368) (369) (370). Similar results regarding prognostic benefit in patients with HFpSF have been reported for beta-blockers (371) although adequate trial data are not currently available. The higher prevalence of patients with HFpSF and probable treatment of these patients according to evidenced-based practice might explain the lack of treatment with a RAAS inhibitor and the lower prescription of beta blockers in the group studied in this chapter. Moreover, lower prevalence of ischaemic heart disease (as reflected by less angina) and diabetes in this group, underlying diseases in which treatment with an ACE inhibitor or ARB is beneficial, might further explain the lack of treatment with RAAS inhibitors in some of these patients. However, the possibility that ischaemic heart disease and diabetes had an impact on the decision with regards to treatment with a RAAS inhibitor might apply mainly to patients with HFpSF, as patients with HFrSF benefit from RAAS inhibitors irrespective of the underlying disease. On the other hand, kidney function was worse in patients not taking a RAAS inhibitor at follow-up. It is likely that a number of patients in this group never started taking a RAAS due to kidney dysfunction or were commenced on a RAAS inhibitor after hospital admission but the treatment was discontinued due to worsening kidney function or electrolyte disturbances. Finally, hypotension or other less frequent side effects might also played a role

in the decision towards no treating some of the patients studied in this chapter with a RAAS inhibitor.

Similar to the patients not treated with a RAAS inhibitor during hospital admission, aldosterone levels were higher in patients with higher PRC at the follow-up visit, indicating that renin angiotensin system continues to play a principal role in the regulation of aldosterone secretion in patients with stable HF. Patients with higher aldosterone levels also had higher cortisol levels after discharge. That is in agreement with previous findings (72) and could indicate that the HPA axis plays a role in the aldosterone secretion in patients with chronic HF. However, PRC was also higher in patients with higher cortisol levels in the current study, making it uncertain that there is an independent role of the HPA in aldosterone secretion in these patients. Renin is not involved in glucocorticoid secretion and the higher RAAS activity in patients with higher HPA activity might be viewed more as an association reflecting the severity of HF rather than a direct pathophysiological relationship.

Patients with lower aldosterone to PRC ratio had higher PRC. These patients had features of more severe HF compared with patients with higher aldosterone to PRC ratio, as reflected by the NYHA functional class, the levels of BNP and kidney function markers as well as the prevalence of elevated troponin. Moreover, ischaemic heart disease was more prevalent in this group as indicated by the history of MI and angina. Overall, it appears that a lower aldosterone to renin ratio is more discriminating than a higher PRC in distinguishing patients not taking a RAAS inhibitor according to HF severity. Interestingly, despite the higher renin levels, aldosterone levels were lower in patients with lower aldosterone to renin ratio compared with patients with higher aldosterone to PRC ratio, suggesting that the activation of the renin angiotensin system did not result into a higher aldosterone secretion in the former group. That indicates that potential antagonists might partially counteract the aldosterone

secreting effects of RAAS in that group. Natriuretic peptides have been shown to suppress aldosterone synthase in vitro as well as to inhibit angiotensin II- and ACTH-induced aldosterone secretion in healthy subjects (125) (127) (372). BNP was higher in patients with lower aldosterone to PRC ratio and that may account for the lower aldosterone levels in these patients. These findings suggest that natriuretic peptides may have a more suppressing effect on the downstream rather than the upstream RAAS components and are in accordance with the finding that although aldosterone levels were normal, approximately half of patients at follow-up visit had elevated PRC. Moreover, they imply that in patients not treated with a RAAS inhibitor lower aldosterone to renin ratio may be a better indicator of HF severity than higher PRC, as it encompasses information not only related with the greater RAAS activity, but also with the expansion of the extravascular volume and raised natriuretic peptide levels as reflected by the lower aldosterone levels (in relation to renin) in these patients

7.4.2 Change in RAAS activity from admission to follow-up

At follow-up, patients not taking a RAAS inhibitor did not on average show activation of RAAS. Almost all patients had aldosterone levels within the normal range and approximately half of patients had normal PRC. However, in the small subset of patients who were not treated with a RAAS inhibitor both prior to admission and after discharge, PRC and aldosterone were higher at follow-up compared with hospital admission, reflecting a greater RAAS activation after discharge. Almost all patients were taking a diuretic at follow-up and this is likely to contribute to the higher RAAS activity. My findings are consistent with a prior study in patients with moderate HF not treated with a RAAS inhibitor or diuretic, in which plasma renin and aldosterone levels were well within the normal range (57). In that study, treatment with low dose furosemide and amiloride for four weeks resulted in a significant increase in both renin and aldosterone levels. Similarly, plasma renin levels were reported to be normal in patients with mild HF and increased following administration of

diuretics (60). Likewise, PRC was elevated only in patients with LVSD with or without HF who were treated with diuretics in the SOLVD study (61).

At follow-up, RAAS activation was present despite the improvement in clinical status and a fall in BNP levels. That is in accordance with previous findings in patients with HF following initiation of diuretic therapy (358) (367) (373) and shows that in my patients the disconnection between BNP and RAAS activity persists at least 4 to 6 weeks after diuretic therapy. Patients lost approximately 4kgs of weight after discharge due to the effective diuresis, which reduces the extracellular volume. That in turn increases RAAS activity and reduces BNP levels. Moreover, as natriuretic peptides suppress the secretion of renin and aldosterone (65), the decline in BNP levels might have additionally contributed to the greater RAAS activity seen 4 to 6 weeks after discharge.

RAAS activation due to diuretics can be detrimental in the long term. Angiotensin II, apart from its vasoconstricting effects, promotes vascular and myocardial remodeling (374) (375). On the other hand aldosterone induces endothelial dysfunction, vascular inflammation and myocardial fibrosis. RAAS activity in patients not receiving an ACE inhibitor or ARB has been associated with worse prognosis in patients with HF (356). Conversely, the inhibition of the RAAS with an ACE inhibitor as monotherapy or in combination with an ARB or an aldosterone blocker has become one of the cornerstones of therapy in patients with HF and LVSD. However, over the course of HF, reactivation of the RAAS might override RAAS inhibition leading to further progression of HF. Thus, in patients with HF an additional treatment approach, which preferably inhibits renin on top of a RAAS inhibitor (and a beta-blocker), might be of additional benefit. Aliskiren, a direct renin inhibitor, exerts favorable effects on neurohumoral activation and is currently being examined with respect to survival benefit, either as an alternative or in combination with another RAAS inhibitor, in patients

with chronic HF (376). Nevertheless, aliskiren on top of standard medical treatment in patients hospitalised with worsening HFrSF had no effect on cardiovascular mortality or HF hospitalisation at 6 or 12 months after discharge in the Aliskiren Trial on Acute Heart Failure Outcomes (ASTRONAUT) (377). Treatment with aliskiren reduced the natriuretic peptide levels, however, that did not translate in better outcomes. On the other hand, aliskiren increased the incidence of adverse events such as hypotension, hyperkalaemia and renal impairment. Interestingly, a subgroup analysis for the composite end point of cardiovascular mortality or HF hospitalisation and also for all-cause mortality at 12 months showed statistically significantly poorer outcomes in diabetics compared with non-diabetics treated with aliskiren. Future studies will reveal if non-diabetics with HF will benefit from add-on therapy with aliskiren in combination with other RAAS inhibitors.

Augmentation of the action of natriuretic peptides might offer another therapeutic option in patients with chronic HF, as these peptides suppress RAAS and SNS besides the diuretic and vasodilating effects. Inhibition of the degradation of natriuretic peptides in combination with an ACE inhibitor was previously examined in HF patients and showed beneficial effects; however, it was not further developed due to adverse effects (angioedema) related to accumulation of bradykinin (378) (379). Blockade of natriuretic peptides breakdown in combination with an ARB instead of an ACE inhibitor is being currently tested in patients with HF and might provide an additional treatment approach (380).

8. Chapter Eight - 11-deoxycortisol and cortisol levels during hospital admission in patients not taking a RAAS inhibitor or oral glucocorticoid therapy

8.1 Introduction

The glucocorticoid hormone cortisol, which plays a pivotal role in metabolism, inflammation and immunity has been increasingly recognised to participate in cardiovascular processes (381) and has also been associated with cardiovascular mortality in the general population (319). In the cardiovascular spectrum, the secretion of cortisol has been mainly studied in patients with hypertension and MI (320) (382). In patients with HF, cortisol has been independently associated with all-cause mortality, cardiovascular mortality or hospitalisation for HF (72) (73). Although the above relationships are generally considered to reflect the stress response in the context of the severity of HF, it has additionally been suggested that cortisol is involved in the pathophysiology of HF progression (section 1.5.1). The latter may gain additional importance in patients with HF as previous studies showed that aldosterone blockers increase cortisol levels in these patients (383).

Despite the increasing evidence about the importance of cortisol in the prognosis and pathophysiology of HF, the secretion of glucocorticoids, has not been extensively examined, especially in relation to the RAAS and other components of the neurohumoral activation, in patients with HF. The main aim of this chapter is to examine the characteristics and markers of HF severity, including RAAS mediators, according to glucocorticoid levels in patients admitted with decompensated HF. The glucocorticoids measured were plasma 11-deoxycortisol and cortisol. The 11-deoxycortisol to cortisol ratio was additionally calculated. Taking into account the inhibiting effects of oral glucocorticoids on cortisol levels and previous studies reporting that aldosterone blockers increase cortisol levels, I have included patients taking none of an oral glucocorticoid or a RAAS inhibitor in this study. In this way, the confounding effect of RAAS inhibitors on the levels of RAAS mediators has also been removed in the analyses of RAAS activity according to glucocorticoid levels in these patients.

8.2 Methods

8.2.1 Study participants and laboratory measurements

Details of the study participants and the laboratory measurements were presented previously in sections 2.3.1 & 2.3.2. Only patients not taking a RAAS inhibitor (ACE inhibitor/ARB or aldosterone blocker) or oral glucocorticoid therapy prior to hospitalisation were included in the current study. All patients had blood samples taken during morning hours between 8-11am.

8.2.2 Statistical analysis

All patient characteristics were expressed as median (IQR) for continuous and as absolute number (percentage) for categorical variables. The inter-group comparisons were carried out by the Mann-Whitney and Kruskal-Wallis test for continuous variables as appropriate and by the χ^2 test for categorical variables. A p-value <0.05 was considered significant for all analyses. Statistical analyses were performed with Minitab version 15.

8.3 Results

8.3.1 Patient characteristics during hospital admission stratified by treatment with a RAAS inhibitor or oral glucocorticoid therapy

Of the 722 patients enrolled, 451 were taking a RAAS inhibitor or oral glucocorticoid therapy and 271 were not taking either a RAAS inhibitor or oral glucocorticoid therapy prior to hospital admission (Table 8-1).

Patients not taking a RAAS inhibitor or an oral glucocorticoid were more often female and were less likely to have a history of previous HF, MI, angina, DM, or hypertension compared with patients taking a RAAS inhibitor or an oral glucocorticoid and patients of the overall hospitalised cohort. The weight, urea and creatinine were significantly lower and the pulse rate, SBP and DBP, haemoglobin and cholesterol were significantly higher in the first group compared with the other two groups. Diuretics, beta-blockers and statins were less often prescribed prior to admission in patients not taking a RAAS inhibitor or glucocorticoid therapy.

Table 8-1. Patient characteristics during hospital admission stratified by treatment with a RAAS inhibitor* or oral glucocorticoid therapy.

Variable	Patients not taking a RAAS inhibitor or oral glucocorticoid therapy (n=271)	Patients taking a RAAS inhibitor or oral glucocorticoid therapy (n=451)	Overall cohort - hospital (n=722)	p-value¶	p-value†
Age (years)	75 (67 - 82)	74 (68 - 80)	74 (68 - 81)	0.473	0.628
Female gender	142 (52)	190 (42.3)	332 (46)	**0.011**	0.089
NYHA class					
II	70 (25.8)	104 (23.1)	174 (24.1)	0.399	0.533
III	163 (60.2)	272 (60.3)	435 (60.3)	0.965	0.977
IV	38 (14.0)	75 (16.6)	113 (15.7)	0.350	0.524
Medical history					
HF	70 (25.8)	250 (55.4)	320 (44.3)	**<0.001**	**<0.001**
MI	88 (32.5)	234 (51.9)	322 (44.6)	**<0.001**	**0.001**
Angina	117 (43.2)	279 (61.9)	396 (54.9)	**<0.001**	**<0.001**
Diabetes mellitus	46 (17.0)	181 (40.1)	227 (31.4)	**<0.001**	**<0.001**
Hypertension	154 (56.3)	324 (71.8)	478 (66.2)	**<0.001**	**0.006**
AF	138 (50.9)	249 (55.2)	387 (53.6)	0.263	0.451
CVA/TIA	52 (19.2)	103 (22.8)	155(21.5)	0.247	0.431
Physiological measurements					
BMI (kg/m^2)	27.1 (22.7 - 31.6)	28.6 (24.8 - 34.1)	27.9 (24 – 32.9)	**<0.001**	**0.007**
Weight (kg)	71.3 (59.4 - 87.3)	77.9 (64.5 - 92)	76 (62.2 - 90)	**<0.001**	**0.011**

Variable	Patients not taking a RAAS inhibitor or oral glucocorticoid therapy (n=271)	Patients taking a RAAS inhibitor or oral glucocorticoid therapy (n=451)	Overall cohort - hospital (n=722)	p-value¶	p-value†
Pulse rate (bpm)	94 (76 - 110)	82 (69 - 100)	86 (71.8 - 106)	**<0.001**	**0.001**
SBP (mmHg)	137 (119 - 155)	130 (114 - 150)	134 (115 - 152)	**0.001**	**0.028**
DBP (mmHg)	80 (66 - 92)	71 (60 – 85)	75 (62 - 88)	**<0.001**	**0.002**
Signs of fluid congestion					
Elevated JVP	189 (78.1)	323 (80.2)	512 (79.4)	0.533	0.676
Peripheral oedema	197 (72.2)	345 (76.8)	542 (75.1)	0.159	0.349
ECG rhythm					
SR	152 (56.1)	246 (54.6)	398 (55.1)	0.686	0.785
AF	110 (40.6)	184 (40.8)	294 (40.7)	0.956	0.970
Echocardiography measurements					
LVEDD (cm)	5.1 (4.6 - 5.8)	5.2 (4.7 - 6.0)	5.2 (4.6 - 5.9)	0.089	0.282
Dilated left ventricle	75 (34.3)	116 (39.6)	191 (37.3)	0.216	0.431
LVH	94 (43.1)	132 (45.4)	226 (44.4)	0.615	0.750
LVSD	150 (68.5)	191 (65.2)	341 (66.6)	0.433	0.618
Laboratory measurements (blood)					
BNP (pg/ml)	874 (417 - 1976)	867 (371 - 1744)	871 (390.8 – 1819.3)	0.234	0.422
Troponin I ≥ 0.04 (µg/L)**	129 (57.9)	201 (53.6)	330 (55.2)	0.312	0.494
Sodium (mmol/L)	138 (135 - 141)	138 (135 - 141)	138 (135 - 141)	0.875	0.916

Variable	Patients not taking a RAAS inhibitor or oral glucocorticoid therapy (n=271)	Patients taking a RAAS inhibitor or oral glucocorticoid therapy (n=451)	Overall cohort - hospital (n=722)	p-value¶	p-value†
Potassium (mmol/L)	4.1 (3.8 - 4.5)	4.2 (3.8 - 4.5)	4.2 (3.8 - 4.5)	0.501	0.650
Urea (mmol/L)	8 (6 - 10.8)	9.4 (6.7 - 13.1)	8.7 (6.3 - 12)	**<0.001**	**0.011**
Creatinine (µmol/L)	99 (82 - 128)	111 (88 -141)	106.5 (85 - 137)	**0.001**	**0.028**
eGFR (ml/min/1.73m^2)	59 (44 - 60)	55 (40 - 60)	56 (41- 60)	**0.041**	0.168
eGFR <60ml/min/1.73m^2	140 (51.7)	265 (58.8)	405 (56.1)	0.063	0.211
Cholesterol (total) (mmol/L)	4.1 (3.4 - 4.9)	3.6 (3.0 - 4.3)	3.7 (3.1 - 4.6)	**<0.001**	**0.003**
HDL (mmol/L)	1.0 (0.8 - 1.4)	1.0 (0.8 - 1.3)	1.0 (0.8 – 1.3)	0.243	0.426
CRP (mg/L)	13 (6.1 - 32)	13 (5.5 - 31)	13 (5.7 - 32)	0.402	0.572
TSH (mIU/L)	1.8 (1.2 - 2.8)	1.7 (1.0 - 2.8)	1.7 (1.0 - 2.8)	0.210	0.395
Cortisol (nmol/L)	336.4 (246.5 - 450)	313.7 (217.1 - 439.5)	322.6 (225.2 - 444.5)	0.133	0.307
11-deoxycortisol (pmol/L)	493.6 (290.1 – 946.5)	492.6 (256 - 926)	492.6 (275.4 - 931.2)	0.639	0.749
11-deoxycortisol/cortisol (10^{-3})	1.64 (1.0 - 2.88)	1.65 (0.96 - 2.91)	1.65 (0.96 - 2.89)	0.866	0.909
Aldosterone (pmol/L)	80.6 (42.1 - 188.1)	66.7 (26.6 – 135.1)	72.3 (31.7 - 154.6)	**0.001**	**0.029**
PRC (mIU/L)	28.2 (9.4 - 79.1)	69.4 (16.6 – 356.7)	47.3 (12.9 - 178.7)	**<0.001**	**<0.001**
Aldosterone/PRC	3.00 (1.08 - 7.51)	0.85 (0.18 - 3.46)	1.54 (0.30 - 4.99)	**<0.001**	**<0.001**
Haemoglobin (g/dl)	12.5 (10.8 - 14)	11.9 (10.4 - 13.2)	12.1 (10.6 - 13.5)	**0.001**	**0.032**
Cardiovascular medication					
Diuretic	139 (51.3)	359 (79.6)	498 (69)	**<0.001**	**<0.001**

Variable	Patients not taking a RAAS inhibitor or oral glucocorticoid therapy (n=271)	Patients taking a RAAS inhibitor or oral glucocorticoid therapy (n=451)	Overall cohort - hospital (n=722)	p-value¶	p-value†
Beta-blocker	99 (36.5)	247 (54.8)	346 (47.9)	**<0.001**	**0.001**
Digoxin	33 (12.2)	84 (18.6)	117 (16.2)	**0.023**	0.114
Anti-arrhythmic	11 (4.1)	18 (4.0)	29 (4.0)	0.964	0.976
Aspirin	127 (46.9)	261 (57.9)	388 (53.7)	**0.004**	0.053
Statin	130 (48)	341 (75.6)	471 (65.2)	**<0.001**	**<0.001**

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).

*ACE inhibitor/ ARB or aldosterone blocker.

** measured at WIG and GRI .

¶ Patients not taking a RAAS inhibitor or an oral glucocorticoid (n=271) vs patients taking a RAAS inhibitor or an oral glucocorticoid (n=451), Mann-Whitney test for continuous and χ^2 test for categorical variables.

† Patients not taking a RAAS inhibitor or an oral glucocorticoid (n=271) vs patients of the overall hospitalised cohort (n=722), Mann-Whitney test for continuous and χ^2 test for categorical variables.

8.3.2 Levels of glucocorticoids during hospital admission

Levels of plasma 11-deoxycortisol and cortisol, and the 11-deoxycortisol to cortisol ratio, during hospital admission are presented below.

8.3.2.1 11-deoxycortisol

An 11-deoxycortisol level measured during hospital admission was available in 220 of the 271 patients. The median (IQR) 11-deoxycortisol was 493.6 (290.1 – 946.5) pmol/L (Figure 8-1) and the mean (SD) 11-deoxycortisol was 806.8 (977) pmol/L. A frequency distribution histogram of 11-deoxycortisol levels in these patients is displayed in Figure 8-2. The minimum 11-deoxycortisol concentration was 23.4 pmol/L and the maximum 11-deoxycortisol concentration was 7008.7 pmol/L. The majority of patients (91%) had 11-deoxycortisol levels within the normal range (0 – 2017 pmol/L).

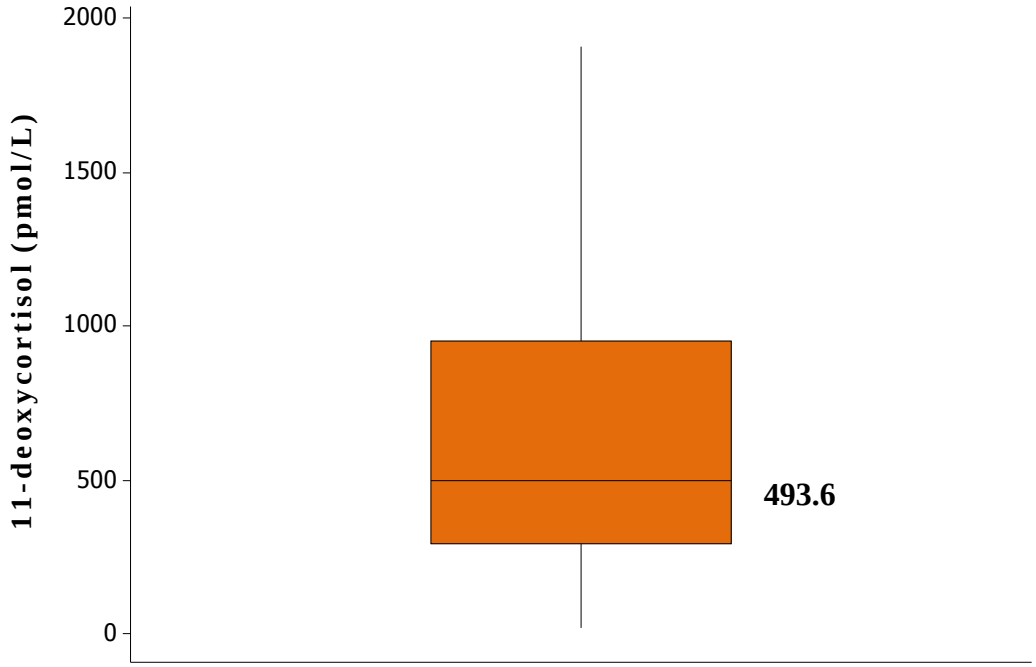


Figure 8-1. Box and whisker plot of 11-deoxycortisol concentrations showing the 2.5, 25, 50, 75 and 97.5 centiles

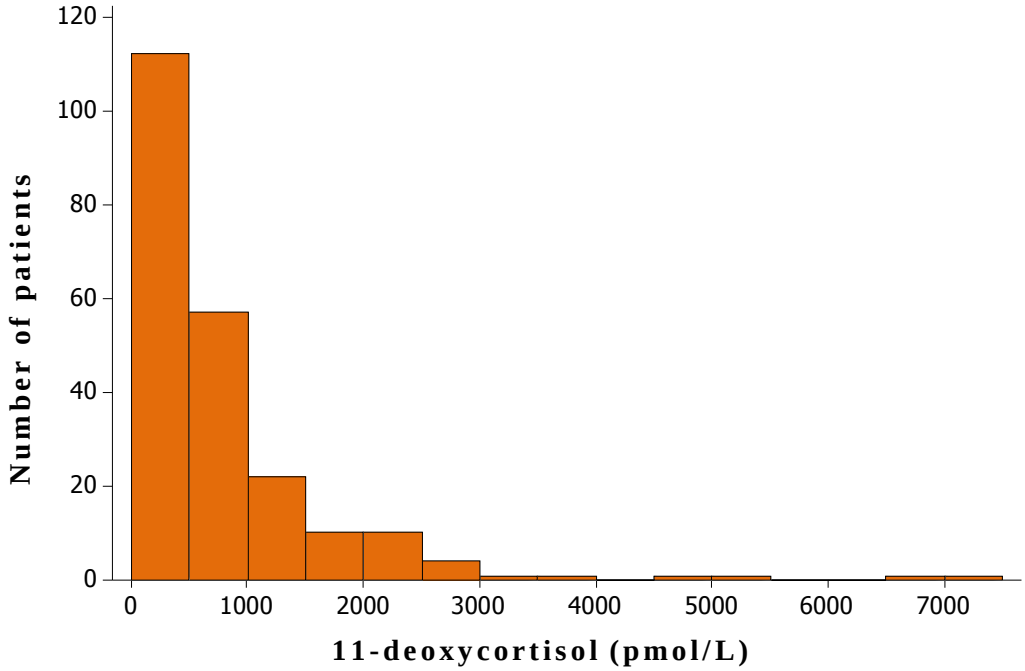


Figure 8-2. Frequency distribution histogram of 11-deoxycortisol concentrations

8.3.2.2 Cortisol

A cortisol level measured during hospital admission was available in 225 of the 271 patients. The median (IQR) cortisol was 336.4 (246.5 – 450.0) nmol/L (Figure 8-3) and the mean (SD) cortisol was 354.6 (158.2) nmol/L. A frequency distribution histogram of cortisol levels in these patients is displayed in Figure 8-4. The minimum cortisol concentration was 6.6 nmol/L and the maximum cortisol concentration was 757.1 nmol/L. All patients (100%) had cortisol levels within the normal range (0 – 823nmol/L).

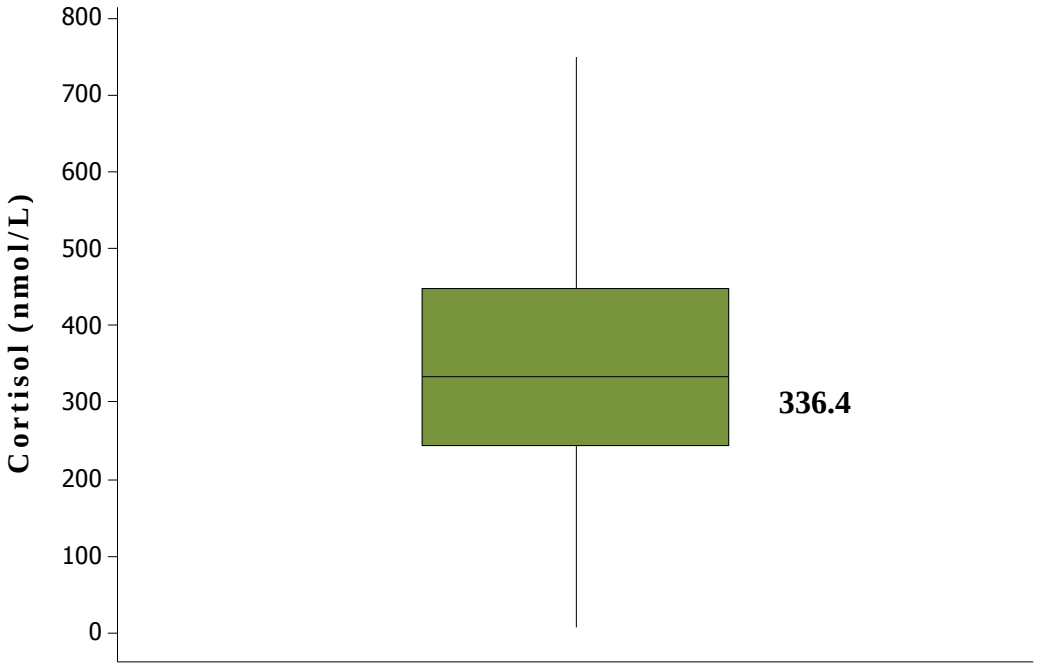


Figure 8-3. Box and whisker plot of cortisol concentrations showing the 2.5, 25, 50, 75 and 97.5 centiles

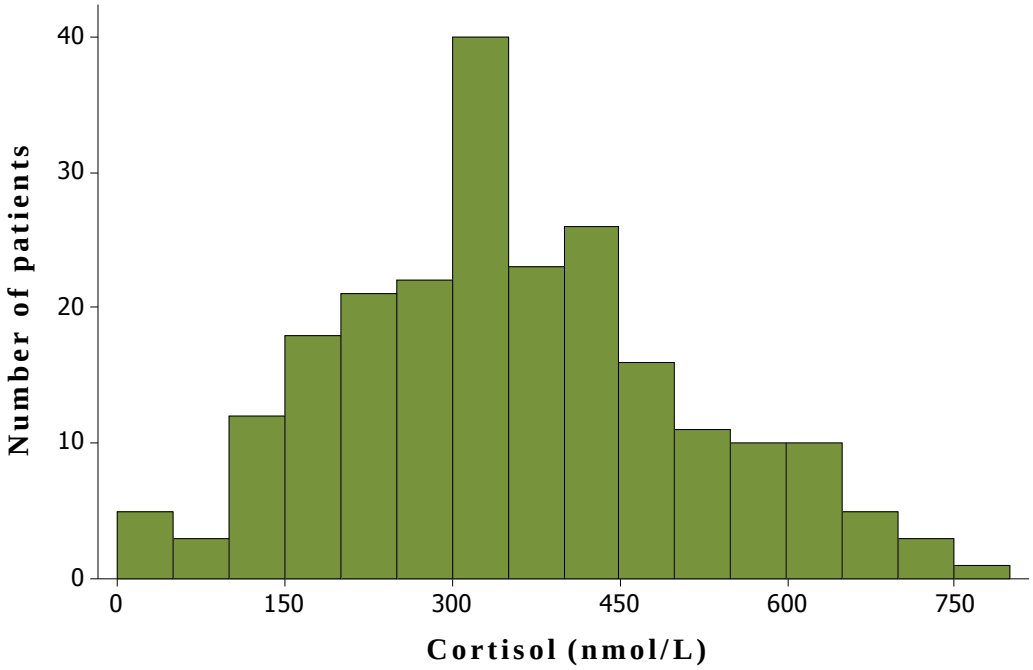


Figure 8-4. Frequency distribution histogram of cortisol concentrations

8.3.2.3 11-deoxycortisol to cortisol ratio

An 11-deoxycortisol to cortisol ratio could be calculated for 220 of the 271 patients studied. The median (IQR) 11-deoxycortisol to cortisol ratio was 1.64 (0.98 – 2.88) $x10^{-3}$ (Figure 8-5) and the mean (SD) 11-deoxycortisol to cortisol ratio was 2.32 (2.39) $x10^{-3}$. A frequency distribution histogram of 11-deoxycortisol to cortisol ratio in these patients is displayed in Figure 8-6. The minimum 11-deoxycortisol to cortisol ratio was 0.07 $x10^{-3}$ and the maximum 11-deoxycortisol to cortisol ratio was 22.33 $x10^{-3}$.

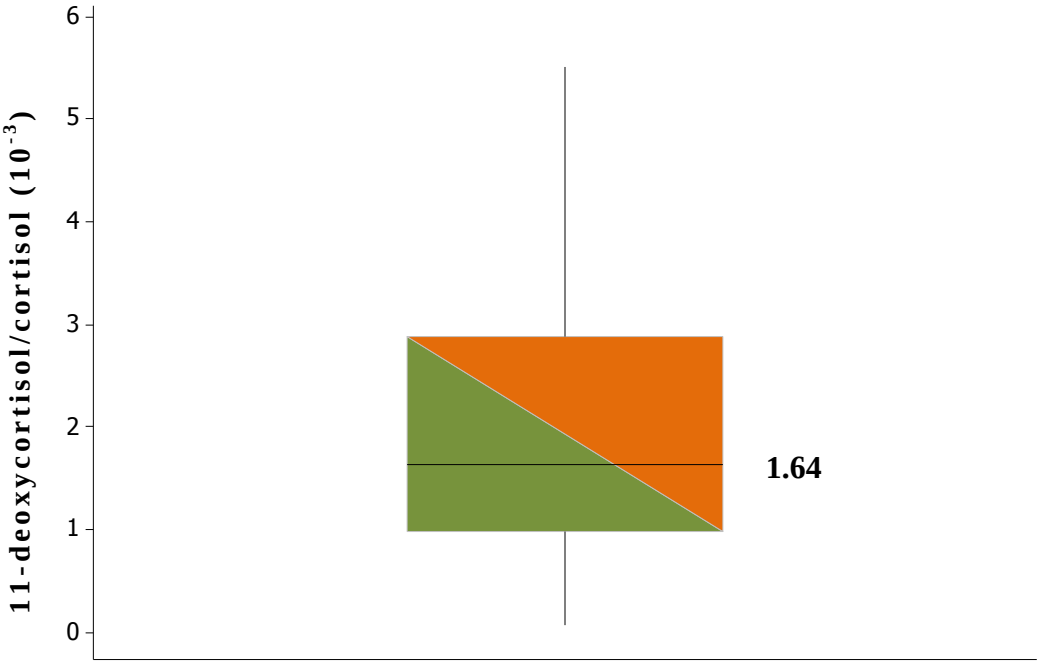


Figure 8-5. Box and whisker plot of 11-deoxycortisol to cortisol ratio showing the 2.5, 25, 50, 75 and 97.5 centiles

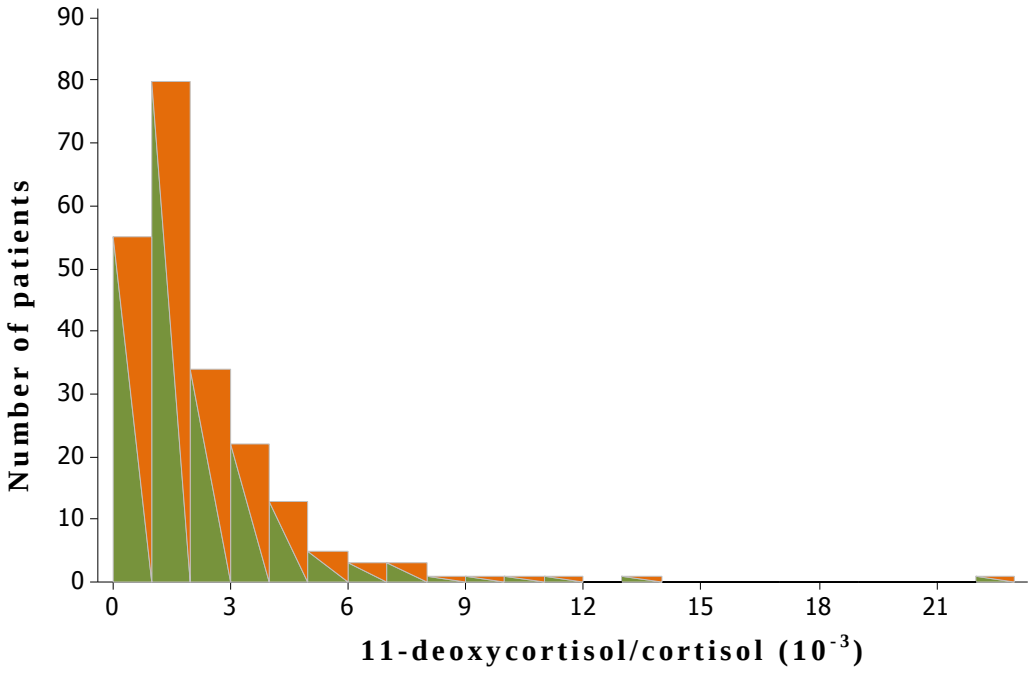


Figure 8-6. Frequency distribution histogram of 11-deoxycortisol to cortisol ratio

8.3.3 Patient characteristics according to glucocorticoid levels

The characteristics of the 271 patients not taking a RAAS inhibitor or oral glucocorticoid therapy were stratified according to the levels of 11-deoxycortisol and cortisol and the 11-deoxycortisol to cortisol ratio during hospital admission.

8.3.3.1 Patient characteristics according to 11-deoxycortisol levels

The group of 220 patients with measured 11-deoxycortisol levels was divided into four subgroups, according to the median 11-deoxycortisol and the 25^{th} and 75^{th} centiles. Such quartiles were respectively defined by 11-deoxycortisol levels <290.1 pmol/L, 290.1 to 493.5 pmol/L, 493.6 to 946.4 pmol/L and ≥ 946.5 pmol/L (Table 8-2).

Compared with those in the highest 11-deoxycortisol quartile, participants in the lowest quartile were more likely to have lower cortisol, 11-deoxycortisol to cortisol ratio and lower SBP. Patients in the lowest 11-deoxycortisol quartile were also more likely to have dilated left ventricle and larger LVEDD and were less likely to have LVH on the transthoracic echocardiogram. In addition, a trend for higher PRC and BNP and lower aldosterone was evident in these patients compared to patients with higher 11-deoxycortisol concentrations.

Table 8-2. Characteristics of patients not taking a RAAS inhibitor* or oral glucocorticoid therapy according to 11-deoxycortisol quartiles

Variable	Q1 (n=55)	Q2 (n=55)	Q3 (n=55)	Q4 (n=55)	p-value†
Age (years)	73 (62 – 80)	75 (64 – 83)	75 (67 – 82)	76 (69 – 86)	0.304
Female gender	32 (58.2)	31 (55.4)	26 (47.3)	27 (49.1)	0.625
NYHA class					
II	16 (29.1)	14 (25.5)	16 (29.1)	15 (27.3)	0.969
III	31 (56.4)	37 (67.3)	31 (56.4)	28 (50.9)	0.364
IV	8 (14.6)	4 (7.3)	8 (14.6)	12 (21.8)	0.197
Medical history					
HF	11 (20)	18 (32.7)	13 (23.6)	16 (29.1)	0.438
MI	11 (20)	18 (32.7)	19 (34.6)	21 (38.2)	0.188
Angina	21 (38.2)	25 (45.5)	25 (45.5)	23 (41.8)	0.845
Diabetes mellitus	12 (21.8)	9 (16.4)	6 (10.9)	14 (25.5)	0.221
Hypertension	28 (50.9)	33 (60)	33 (60)	33 (60)	0.706
AF	23 (41.8)	30 (54.6)	29 (52.7)	32 (58.2)	0.351
CVA/TIA	6 (10.9)	12 (21.8)	10 (18.2)	15 (27.3)	0.176
Physiological measurements					
BMI (kg/m^2)	24.6 (22.1 – 30.1)	28.0 (24.4 – 31.5)	26.5 (22.7 - 32.8)	27.1 (22.6 – 33.8)	0.358
Pulse rate (bpm)	93 (77 – 110)	96 (80 – 110)	94 (72 - 116)	88 (70 - 104)	0.326
SBP (mmHg)	130 (114 – 140)	140 (118 – 160)	140 (125 – 162)	138 (122 – 156)	**0.008**
DBP (mmHg)	72 (64 – 83)	81 (66 – 95)	86 (75 – 92)	77 (62 – 95)	**0.020**

Variable	Q1 (n=55)	Q2 (n=55)	Q3 (n=55)	Q4 (n=55)	p-value†
Signs of fluid congestion					
Elevated JVP	36 (73.5)	40 (80)	37 (78.7)	42 (82.4)	0.740
Peripheral oedema	39 (70.9)	44 (78.6)	41 (75.6)	39 (70.9)	0.760
ECG rhythm					
SR	32 (58.2)	29 (52.7)	30 (54.6)	30 (54.6)	0.951
AF	21 (38.2)	23 (41.8)	22 (40.0)	24 (43.6)	0.945
Echocardiography measurements					
LVEDD (cm)	5.6 (4.9 – 6.2)	5.2 (4.4 – 5.6)	5.1 (4.6 – 5.9)	5.0 (4.5 – 5.5)	**0.014**
Dilated left ventricle	24 (54.6)	16 (34.8)	16 (34.8)	10 (23.8)	**0.028**
LVH	11 (25)	24 (53.3)	22 (47.8)	22 (52.4)	**0.024**
LVSD	31 (70.5)	27 (58.7)	34 (73.9)	27 (64.3)	0.424
Laboratory measurements (blood)					
BNP (pg/ml)	1032 (531 – 2193)	841 (365 – 1512)	956 (405 – 1869)	694 (373 – 2010)	0.732
Troponin I ≥ 0.04 (µg/L)**	25 (54.4)	27 (57.5)	27 (60.0)	27 (58.7)	0.955
Sodium (mmol/L)	137 (133 – 141)	138 (135 – 140)	139 (136 – 141)	138 (135 – 141)	0.606
Potassium (mmol/L)	4.1 (3.9 – 4.5)	4.2 (3.9 – 4.5)	4.0 (3.8 – 4.3)	4.1 (3.7 – 4.6)	0.517
Urea (mmol/L)	8.0 (6.2 – 10.7)	7.5 (5.0 – 11.0)	8.4 (5.9 – 10.7)	8.1 (6.0 – 11.8)	0.540
Creatinine (µmol/L)	97 (79 – 130)	101 (85 – 133)	108 (79 – 139)	97 (82 – 129)	0.867
eGFR (ml/min/1.73m^2)	60 (42 – 60)	56 (40 – 60)	57 (44 – 60)	60 (43 – 60)	0.763
eGFR <60ml/min/1.73m^2	27 (49.1)	33 (60)	30 (54.6)	26 (47.3)	0.534

Variable	Q1 (n=55)	Q2 (n=55)	Q3 (n=55)	Q4 (n=55)	p-value†
Cholesterol (total) (mmol/L)	4.2 (3.5 – 4.9)	4.3 (3.5 – 5.0)	3.7 (3.4 - 4.4)	4.4 (3.2 – 5.2)	0.336
HDL (mmol/L)	1.0 (0.8 – 1.4)	1.1 (0.9 – 1.4)	1.0 (0.8 – 1..2)	1.1 (0.9 – 1.5)	0.605
CRP (mg/L)	14 (7 – 38)	12 (7 – 29)	14 (6 – 26)	14.5 (6 – 48)	0.787
Cortisol (nmol/L)	265.5(141.3 – 330.9)	305.8 (219.1 – 389.4)	362.7 (304.4 – 456.0)	460.9 (392.2 – 595.9)	**<0.001**
11-deoxycortisol/cortisol (10^{-3})	0.72 (0.46 – 1.11)	1.35 (0.96 – 1.73)	1.68 (1.36 – 2.52)	3.72 (2.85 – 4.88)	**<0.001**
Aldosterone (pmol/L)	62.0 (28.4 – 169.2)	80.1 (39.0 – 190.0)	100.6 (51.0 – 171.4)	78.4 (51.9 – 222.1)	0.509
PRC (mIU/L)	39.5 (12.4 – 91.0)	28.9 (10.4 – 103.3)	18.9 (9.0 – 48.9)	23.25 (7.9 – 74.8)	0.215
Aldosterone/PRC	2.71 (0.58 – 6.17)	2.35 (0.91 - 4.51)	3.98 (1.35 – 10.66)	4.0 (1.47 – 10.20)	0.051
TSH (mIU/L)	2.0 (0.9 – 3.3)	1.8 (1.3 – 2.5)	1.9 (1.2 – 3.0)	1.6 (1.0 – 2.1)	0.532
Haemoglobin (g/dl)	12.2 (10.4 – 13.4)	13.0 (11.5 – 14.5)	12.5 (10.7 – 14.1)	12.5 (10.7 – 13.6)	0.239
Cardiovascular medication prior to admission					
Diuretic	30 (54.6)	30 (54.6)	29 (52.7)	29 (52.7)	0.995
Beta-blocker	16 (29.1)	20 (36.4)	22 (40.0)	26 (47.3)	0.261
Digoxin	5 (9.1)	12 (21.8)	5 (9.1)	9 (16.4)	0.156
Anti-arrhythmic	0 (0)	3 (5.5)	4 (7.3)	3 (5.5)	0.287
Aspirin	21 (38.2)	30 (54.6)	24 (43.6)	29 (52.7)	0.268
Statin	22 (40)	27 (49.1)	28 (50.9)	28 (50.9)	0.614

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).
* ACE inhibitor/ARB or aldosterone blocker
† Kruskal-Wallis test was used for continuous variables and χ^2 test for categorical variables.
**measured at WIG and GRI

8.3.3.2 Patient characteristics according to cortisol levels

The group of 225 patients with measured cortisol levels was divided into four subgroups, according to the median cortisol and the 25th and 75th centiles. Such quartiles were respectively defined by cortisol levels <246.5 nmol/L, 246.5 to 336.3 nmol/L, 336.4 to 449.9 nmol/L and ≥ 450.0 nmol/L (Table 8-3).

Compared with those in the lowest cortisol quartile, participants in the highest quartile were more likely to have higher 11-deoxycortisol. Patients in the highest cortisol quartile were also more likely to have elevated troponin I compared with patients in the lowest cortisol quartile. Apart from the above differences, a trend for higher BNP, urea, creatinine and CRP and smaller LVEDD was present in patients with higher cortisol levels. These patients were also more often in NYHA class IV and less often in NYHA class II compared to patients with lower cortisol levels.

Table 8-3. Characteristics of patients not taking a RAAS inhibitor* or oral glucocorticoid therapy according to cortisol quartiles

Variable	Q1 (n=56)	Q2 (n=56)	Q3 (n=57)	Q4 (n=56)	p-value†
Age (years)	71 (62.3 – 79.8)	77 (71 – 83)	75 (66.5 – 81)	76.5 (66.3 – 84.8)	0.134
Female gender	32 (57.1)	32 (56.1)	24 (42.1)	32 (57.1)	0.294
NYHA class					
II	20 (35.7)	18 (32.1)	12 (21.1)	11 (19.6)	0.141
III	29 (51.8)	35 (62.5)	36 (63.2)	32 (57.1)	0.581
IV	7 (12.5)	3 (5.4)	9 (15.8)	13 (23.2)	0.056
Medical history					
HF	15 (26.8)	15 (26.8)	17 (29.8)	13 (23.2)	0.889
MI	21 (37.5)	19 (33.9)	15 (26.3)	17 (30.4)	0.617
Angina	24 (42.9)	28 (50.0)	23 (40.4)	22 (39.3)	0.606
Diabetes mellitus	8 (14.3)	6 (10.7)	14 (24.6)	13 (23.2)	0.159
Hypertension	31 (55.4)	33 (58.9)	33 (57.9)	33 (58.9)	0.978
AF	24 (42.9)	30 (53.6)	28 (49.1)	34 (60.7)	0.283
CVA/TIA	10 (17.9)	11 (19.6)	10 (17.5)	13 (23.2)	0.867
Physiological measurements					
BMI (kg/m^2)	28 (22.4 – 33.4)	26.2 (23.9 – 30.9)	28.4 (23.4 – 33)	24.7 (20.9 – 30.7)	**0.038**
Pulse rate (bpm)	92 (78 – 109.5)	89 (73.3 – 116.5)	94 (72 – 105.5)	91 (75.5 – 110)	0.888
SBP (mmHg)	137.5 (115 – 150)	134.5 (122 – 150.5)	135 (120.5 – 158)	137.5 (115 – 154.8)	0.936
DBP (mmHg)	75 (65.5 – 91)	78 (66.3 – 86)	82 (69 – 94)	81 (62.8 – 94.5)	0.461

Variable	Q1 (n=56)	Q2 (n=56)	Q3 (n=57)	Q4 (n=56)	p-value†
Signs of fluid congestion					
Elevated JVP	34 (75.6)	45 (83.3)	36 (69.2)	44 (86.3)	0.139
Peripheral oedema	39 (69.4)	46 (80.7)	43 (75.4)	38 (67.9)	0.397
ECG rhythm					
SR	33 (58.9)	27 (48.2)	35 (61.4)	29 (51.8)	0.463
AF	21 (37.5)	27 (48.2)	18 (31.6)	26 (46.4)	0.234
Echocardiography measurements					
LVEDD (cm)	5.3 (4.6 – 6.1)	5.1 (4.7 – 5.7)	5.2 (4.6 – 5.8)	4.8 (4.5 – 5.9)	0.497
Dilated left ventricle	16 (34.8)	16 (36.4)	19 (42.2)	15 (32.6)	0.802
LVH	22 (47.3)	17 (39.5)	22 (48.9)	18 (39.1)	0.682
LVSD	26 (56.5)	29 (65.9)	36 (80)	31 (67.4)	0.123
Laboratory measurements (blood)					
BNP (pg/ml)	638.5 (329 – 1262)	853.5 (369 – 2002)	1236 (518 – 2821)	975.5 (552 – 2355)	**0.045**
Troponin I ≥ 0.04 (µg/L)**	23 (47.9)	21 (44.7)	32 (68.1)	34 (72.3)	**0.010**
Sodium (mmol/L)	138 (135 – 141)	139 (136 – 141)	138 (135 – 140)	137 (135 – 140)	0.225
Potassium (mmol/L)	4.2 (3.9 – 4.5)	4.1 (3.6 – 4.4)	4.1 (3.9 – 4.5)	4.2 (3.7 – 4.7)	0.437
Urea (mmol/L)	6.8 (5.8 – 9.0)	8.1 (6.0 – 11.1)	8.7 (5.9 – 10.7)	8.3 (6.1 – 13.3)	0.061
Creatinine (µmol/L)	94.5 (78.3 – 115)	103.5 (82.3 – 135)	101 (83.5 – 124)	108.5 (80 – 140)	0.419
eGFR (ml/min/1.73m^2)	60 (47.5 – 60)	57.5 (42 – 60)	59 (41 – 60)	56 (36.5 – 60)	0.593
eGFR <60ml/min/1.73m^2	27 (48.2)	30 (53.6)	29 (50.9)	32 (57.1)	0.806

Variable	Q1 (n=56)	Q2 (n=56)	Q3 (n=57)	Q4 (n=56)	p-value†
Cholesterol (total) (mmol/L)	4.0 (3.5 – 4.7)	4.1 (3.1 – 5.0)	3.8 (3.5 – 4.9)	4.4 (3.2 – 5.3)	0.853
HDL (mmol/L)	1.1 (0.9 – 1.4)	1.0 (0.8 – 1.4)	1.0 (0.9 – 1.3)	1.0 (0.9 – 1.4)	0.866
CRP (mg/L)	11 (5.9 – 36.5)	13.5 (6.7 – 28)	13 (5.5 – 25)	15 (8.2 – 53.8)	0.339
11-deoxycortisol (pmol/L)	302.7 (168.8 – 421)	430.6 (276.7 – 801.7)	541.8 (339.8 – 910.6)	1097.0 (519.0 – 2226.0)	**<0.001**
11-deoxycortisol/cortisol (10^{-3})	1.8 (1.30 – 2.99)	1.49 (0.87 – 2.73)	1.44 (0.89 – 2.36)	1.93 (1.07 – 3.95)	0.072
Aldosterone (pmol/L)	84.2 (41.1 – 221.8)	60.3 (29.7 – 113.1)	101.8 (46.7 – 228.8)	79.4 (55.0 – 241.0)	**0.037**
PRC (mIU/L)	16.8 (8.4 – 49.3)	36.0 (9.0 – 79)	23.9 (6.9 – 94.5)	32.0 (12.0 - 118.5)	0.285
Aldosterone/PRC	4.58 (2.19 – 9.67)	1.51 (0.74 - 5.29)	2.96 (1.58 – 7.33)	3.38 (1.09 – 10.27)	**0.036**
TSH (mIU/L)	1.8 (1.3 – 2.5)	1.9 (1.0 – 3.5)	1.7 (1.1 – 2.5)	1.6 (1.1 – 3.1)	0.927
Haemoglobin (g/dl)	12.4 (11.2 – 14.0)	13.0 (11.6 – 14.4)	12.2 (10.1 – 14.1)	11.8 (10.3 – 12.9)	0.103
Cardiovascular medication prior to admission					
Diuretic	31 (55.4)	30 (53.6)	31 (54.4)	27 (48.2)	0.876
Beta-blocker	17 (30.4)	25 (44.6)	24 (42.1)	19 (33.9)	0.356
Digoxin	6 (10.7)	11 (19.6)	8 (14)	6 (10.7)	0.474
Anti-arrhythmic	3 (5.4)	0 (0)	5 (8.8)	2 (3.6)	0.149
Aspirin	28 (50)	28 (50)	26 (45.6)	25 (44.6)	0.909
Statin	29 (51.8)	27 (48.2)	26 (45.6)	27 (48.2)	0.933

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).
* ACE inhibitor/ARB or aldosterone blocker
† Kruskal-Wallis test was used for continuous variables and χ^2 test for categorical variables.
**measured at WIG and GRI

8.3.3.3 Patient characteristics according to 11-deoxycortisol to cortisol ratio

The group of 220 patients with calculated 11-deoxycortisol to cortisol ratio was divided into four subgroups, according to the median 11-deoxycortisol to cortisol ratio and the 25^{th} and 75^{th} centiles. Such quartiles were respectively defined by 11-deoxycortisol to cortisol <0.98 $x10^{-3}$, 0.98 $x10^{-3}$ to 1.63 $x10^{-3}$, 1.64 $x10^{-3}$ to 2.87 $x10^{-3}$ and ≥ 2.88 $x10^{-3}$ (Table 8-4).

Compared with those in the highest 11-deoxycortisol to cortisol ratio quartile, participants in the lowest quartile were more likely to have lower 11-deoxycortisol, aldosterone to PRC ratio, SBP and sodium and higher PRC, BNP and CRP. Patients in the lowest 11-deoxycortisol to cortisol quartile were also more likely to have dilated left ventricle and were less likely to have LVH on the transthoracic echocardiogram. In addition, there was a trend for larger LVEED, higher prevalence of LVSD and elevated troponin in patients with lower 11-deoxycortisol to cortisol ratio.

Table 8-4. Characteristics of patients not taking a RAAS inhibitor* or oral glucocorticoid therapy according to 11-deoxycortisol to cortisol ratio quartiles

Variable	Q1 (n=55)	Q2 (n=55)	Q3 (n=55)	Q4 (n=55)	p-value†
Age (years)	73 (62 – 81)	76 (67 – 83)	73 (66 – 82)	76 (69 – 86)	0.321
Female gender	28 (50.9)	31 (55.4)	28 (50.9)	29 (52.7)	0.961
NYHA class					
II	15 (27.27)	10 (18.18)	20 (36.36)	16 (29.09)	0.203
III	33 (60)	36 (65.5)	27 (49.1)	31 (56.4)	0.364
IV	7 (12.7)	9 (16.4)	8 (14.6)	8 (14.6)	0.961
Medical history					
HF	12 (21.8)	15 (27.27)	16 (29.1)	15 (27.27)	0.839
MI	12 (21.8)	16 (29.1)	17 (30.9)	24 (43.6)	0.097
Angina	21 (38.2)	30 (54.6)	15 (27.27)	28 (50.9)	**0.015**
Diabetes mellitus	10 (18.2)	8 (14.6)	6 (10.9)	17 (30.9)	**0.041**
Hypertension	28 (50.9)	31 (56.4)	31 (56.4)	37 (67.3)	0.364
AF	23 (41.8)	34 (61.8)	28 (50.9)	29 (52.7)	0.218
CVA/TIA	6 (10.9)	9 (16.4)	10 (18.2)	18 (32.7)	**0.028**
Physiological measurements					
BMI (kg/m^2)	25.6 (22.1 – 30.4)	27 (23.3 – 31.3)	27.9 (22.0 – 33.0)	27.4 (23.9 – 35.1)	0.237
Pulse rate (bpm)	93 (80 – 110)	90 (77 – 106)	99 (78 – 120)	88 (68 – 98)	0.072
SBP (mmHg)	131 (114 – 140)	140 (115 – 152)	145 (119 – 170)	137 (124 – 155)	**0.027**

Variable	Q1 (n=55)	Q2 (n=55)	Q3 (n=55)	Q4 (n=55)	p-value†
DBP (mmHg)	76 (62 – 87)	81 (71 – 90)	80 (68 – 100)	77 (65 – 88)	0.256
Signs of fluid congestion					
Elevated JVP	41 (78.9)	37 (75.5)	36 (80)	41 (80.4)	0.934
Peripheral oedema	42 (76.4)	41 (73.2)	38 (69.1)	42 (76.4)	0.798
ECG rhythm					
SR	33 (60)	26 (47.3)	30 (54.6)	32 (58.2)	0.549
AF	20 (36.4)	26 (47.3)	23 (41.8)	21 (38.2)	0.664
Echocardiography measurements					
LVEDD (cm)	5.5 (4.8 – 6.1)	5.1 (4.6 – 5.7)	5.1 (4.5 – 5.9)	5.0 (4.5 – 5.5)	0.151
Dilated left ventricle	26 (54.2)	15 (34.1)	13 (31.7)	12 (26.7)	**0.032**
LVH	14 (29.8)	21 (47.7)	16 (39)	28 (62.2)	**0.015**
LVSD	34 (70.8)	30 (68.2)	30 (73.2)	25 (55.6)	0.295
Laboratory measurements (blood)					
BNP (pg/ml)	1436 (571 – 2273)	1134 (427 – 1832)	639 (339 – 1589)	658 (346 – 1512)	**0.038**
Troponin I ≥ 0.04 (µg/L)**	31 (68.9)	23 (47.9)	29 (64.4)	23 (50)	0.105
Sodium (mmol/L)	137 (134 – 141)	137 (135 – 139)	140 (138 – 142)	138 (135 – 141)	**0.012**
Potassium (mmol/L)	4.1 (3.8 – 4.4)	4.1 (3.9 – 4.6)	4.1 (3.8 – 4.5)	4.1 (3.7 – 4.5)	0.629
Urea (mmol/L)	8.6 (6.3 – 12.5)	8.0 (5.7 – 11.1)	6.9 (5.4 – 10.7)	7.9 (6.4 – 10.9)	0.190
Creatinine (µmol/L)	98 (83 – 143)	108 (85 – 127)	100 (79 – 128)	99 (79 – 127)	0.779
eGFR (ml/min/1.73m^2)	56 (38 – 60)	56 (42 – 60)	59 (44 – 60)	60 (44 – 60)	0.714

Variable	Q1 (n=55)	Q2 (n=55)	Q3 (n=55)	Q4 (n=55)	p-value†
eGFR <60ml/min/1.73m^2	30 (54.6)	32 (58.2)	29 (52.7)	25 (45.5)	0.594
Cholesterol (total) (mmol/L)	4.1 (3.2 – 5.1)	4.0 (3.6 – 4.8)	3.7 (3.4 – 4.5)	4.5 (3.2 – 5.0)	0.566
HDL (mmol/L)	1.0 (0.7 – 1.3)	1.1 (0.9 – 1.4)	1.0 (0.9 – 1.2)	1.2 (0.8 – 1.6)	0.197
CRP (mg/L)	18 (9.9 – 45)	15 (5.7 – 29)	10 (6 – 25)	10 (4.6 – 26)	0.051
Cortisol (nmol/L)	340.3 (277.9 – 441.3)	358.2 (269.9 – 438.6)	304.4 (204.8 – 428.4)	403.8 (228.5 – 482.2)	0.101
11-deoxycortisol (pmol/L)	250.8 (116.0 – 314.4)	479.9 (357.7 – 579.7)	609.3 (375.6 – 914.9)	1517.3 (932.0 – 2256.0)	**<0.001**
Aldosterone (pmol/L)	82.7 (35.0 – 244.0)	81.2 (50.6 – 171.4)	79 (39.1 – 146.5)	78.67 (54.2 – 222.7)	0.707
PRC (mIU/L)	67.7 (23.7 – 103.3)	15.8 (7.5 – 46.8)	19.5 (8.1 – 45.8)	22.0 (8.7 – 67.1)	**0.001**
Aldosterone/PRC	1.64 (0.60 – 4.17)	3.78 (1.33 – 9.16)	2.62 (1.39 – 7.57)	4.33 (1.26 – 10.09)	**0.016**
TSH (mIU/L)	2.0 (1.3 – 2.9)	1.9 (1.1 – 2.7)	1.9 (1.1 – 3.1)	1.6 (1.0 – 1.8)	0.409
Haemoglobin (g/dl)	12.2 (10.3 – 14.0)	12.2 (10.4 – 13.8)	12.9 (11.3 – 14.5)	12.5 (11.2 – 13.6)	0.395
Cardiovascular medication prior to admission					
Diuretic	29 (52.7)	28 (50.9)	28 (50.9)	33 (60)	0.743
Beta-blocker	16 (29.1)	21 (38.2)	23 (41.8)	24 (43.6)	0.403
Digoxin	4 (7.3)	11 (20)	10 (18.2)	6 (10.9)	0.178
Anti-arrhythmic	1 (1.8)	2 (3.6)	4 (7.3)	3 (5.5)	0.553
Aspirin	24 (43.6)	25 (45.5)	22 (40)	33 (60)	0.164
Statin	21 (38.2)	26 (47.3)	24 (43.6)	34 (61.8)	0.080

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).
* ACE inhibitor/ARB or aldosterone blocker
† Kruskal-Wallis test was used for continuous variables and χ^2 test for categorical variables.
**measured at WIG and GRI

8.4 Discussion

8.4.1 Patient characteristics according to glucocorticoid secretion during hospital admission

The levels of 11-deoxycortisol, cortisol and the 11-deoxycortisol to cortisol ratio in the subset of patients not taking a RAAS inhibitor or oral glucocorticoid therapy were similar to the relevant glucocorticoid levels and their ratio in the overall hospitalised cohort. The levels of cortisol were higher in patients with higher 11-deoxycortisol concentrations. That is not surprising taking into account that the last step in cortisol synthesis in ZF involves the 11beta- hydroxylation of 11-deoxycortisol, which is the immediate precursor of cortisol in adrenal steroidogenesis (section 1.2.1). Although cortisol levels in my patients were within the so called "normal range", there was a clear gradient in severity of HF according to cortisol levels with patients with higher levels having worse clinical and prognostic features. Patients with higher cortisol were more likely to have elevated troponin during follow-up, which reflects the degree of myocardial necrosis. A direct link between glucocorticoids and myocardial necrosis in patients with HF has not been demonstrated and the above association may reflect the severity of HF in these patients. Indeed, patients in the highest cortisol quartile were more often in NYHA IV and less likely in NYHA class II compared to patients in the lowest cortisol quartile. Correspondingly, patients with higher cortisol levels were more likely to have, apart from elevated troponin, higher BNP and LVSD. In keeping with these findings, cortisol has been previously associated with norepinephrine in patients with HF (72), which is an independent prognostic indicator in HF (384).

Whether some of the relationships between cortisol and established prognostic markers, however, represent a cause-and-effect relationship cannot be excluded. As described in section 1.3.5, glucocorticoids exert deleterious effects on the cardiovascular system either by activating the GRs or through the activation of the MRs under conditions of altered

intracellular redox state. Moreover, it has been suggested that normal cortisol levels are sufficient to activate MR and exert detrimental non-epithelial effects in patients with HF (72). MR activation is associated with peri-vascular inflammation, myocardial necrosis and apoptosis and these effects might represent a possible link for the observed association between cortisol and troponin. In addition, cortisol exerts mineralocorticoid epithelial effects under circumstances of impaired metabolism by 11beta-HSD2 (385) (386). A decline in the expression and activity of 11beta-HSD2 in the kidneys has been reported in patients with chronic kidney disease (387). More than half of patients in my study had eGFR <60 ml/min/1.73m^2; thus, a possible glucocorticoid-induced MR activation in kidneys may lead to an increase in the extravascular volume and potentially to higher natriuretic peptide levels.

Overall, the associations between markers of HF severity and cortisol were present despite the normal on average glucocorticoid levels, providing, thus, the rationale to rethink what we mean by "normal" levels of cortisol in patients with HF.

Interestingly, patients with lower 11-deoxycortisol to cortisol ratio were more likely to have higher PRC. In addition, these patients were more likely to have dilated left ventricle (and less likely to have LVH), representing a group of patients at a more progressed stage of LV remodeling. Moreover, similar to patients with higher PRC (section 6.3.3.2) they had lower SBP and sodium and higher BNP. The reasons for the above findings are not clear. It is generally accepted that 11-deoxycortisol to cortisol ratio represents an index of 11beta-hydroxylase activity and a lower 11-deoxycortisol to cortisol ratio reflects a higher activity of this enzyme; 11-deoxycortisol under these circumstances is utilised more efficiently, with less leakage into the bloodstream, by 11beta-hydroxylase for the formation of the cortisol. Hence, greater amounts of cortisol, are secreted into the circulation in relation to 11-deoxycortisol. 11beta-hydroxylase is an ACTH dependent enzyme and its activity reflects the

HPA axis stimulation. Indeed, chronic ACTH activation has been shown to up-regulate the late phase of cortisol biosynthesis with augmentation in the conversion of 11-deoxycortisol to cortisol (388) (389). Moreover, ACTH exerts trophic effects on 11beta-hydroxylase and also causes adrenocortical cell hypertrophy with increase in the number of mitochondria, where the conversion of 11-deoxycortisol to cortisol takes place (390) (391). In patients with chronic ACTH excess and Cushing disease, however, the levels of cortisol precursors were found to lie within or below the normal range (392). Likewise, in hypophysectomised rats models ACTH enhanced the conversion of DOC to corticosterone, indicating an up-regulation of the 11beta-hydroxylase activity (393). In contrast, acute stimulation with ACTH produces elevation in both cortisol and its precursors (388) (394).

These insights into the physiology of glucocorticoid secretion suggest that patients with higher 11beta-hydroxylase activity, which is likely due to chronic HPA axis activation, had higher RAAS activity, worse LV remodeling and lower blood pressure. It is important to note that the above associations were also present in the overall hospitalised cohort (Table 13-8 in the Appendix). Various pathways might contribute to the HPA activation in these patients. Haemodynamically stressful stimuli, such as low blood pressure, might contribute to ACTH stimulation (395). In addition, a general inflammatory state, as reflected by the higher CRP, is associated with up-regulation of cytokine expression, which in turn can potentially stimulate ACTH secretion (396) (397). Moreover, the SNS also participates in the regulation of cortisol secretion mainly through the autonomic innervation of the adrenal cortex (398). Thus, in patients with HF, apart from RAAS (and the SNS) activation, HPA axis activation may represent an additional pathway of the adaptive neurohumoral responses.

The findings of the current study suggest that inhibition of 11beta-hydroxylase and lowering of cortisol levels might be of benefit in patients with HF. Aldosterone synthase inhibitors

apart from suppressing aldosterone levels, inhibit partially 11beta-hydroxylase (350) (351). The latter effect is not associated with lower cortisol levels but with suppression of ACTH-induced release of cortisol in patients with essential hypertension. That might be of clinical benefit in patients with HF according to the findings in my study, as patients with features of worse HF had higher 11beta-hydroxylase activity, presumably due to chronic ACTH stimulation. Hence, suppression of both mineralocorticoid and glucocorticoid secretion with these agents may provide additional therapeutic opportunities in patients with HF. However, in contrast to the appealing theoretical effects, the safety of these agents may be compromised under acute conditions, where the stress response is clinically useful. A study examining the safety and efficacy of aldosterone synthase inhibitors in patients with HF will provide information with respect to theoretical benefits and concerns.

9. Chapter Nine - 11-deoxycortisol and cortisol levels at follow-up in patients not taking a RAAS inhibitor or oral glucocorticoid therapy

9.1 Introduction

The main aim of this chapter is to examine if the associations among glucocorticoid levels, RAAS activation and markers of HF severity seen in patients with decompensated HF are present in patients with stable HF not taking an oral glucocorticoid and a RAAS inhibitor at the follow-up visit. In this chapter, I also compare the levels of glucocorticoids measured during hospital admission and after discharge in patients not receiving oral glucocorticoid therapy or a RAAS inhibitor at both time points.

9.2 Methods

9.2.1 Study participants and laboratory measurements

Details of the study participants and the laboratory measurements were presented previously in 2.4.1 & 2.4.2. Only patients not receiving a RAAS inhibitor or oral glucocorticoid therapy during follow-up were included in the current study. All blood samples were drawn during afternoon hours between 12 - 4 pm. For the comparisons of glucocorticoid levels between hospital admission and the follow-up visit, the subgroup of patients not receiving a RAAS inhibitor or oral glucocorticoid prior to admission and at follow-up was included in the analyses.

9.2.2 Statistical analysis

All baseline characteristics are expressed as median (IQR) for continuous and absolute number (percentage) for categorical variables. The inter-group comparisons were carried out by the Mann-Whitney and Kruskal-Wallis test for continuous variables as appropriate and by the χ^2 test for categorical variables. For the comparisons of baseline characteristics between hospitalised and post-discharge patients not receiving a RAAS inhibitor or oral

glucocorticoid treatment, Wilcoxon matched pairs test and McNemar test were employed for continuous and categorical variables respectively. A p-value <0.05 was considered significant for all analyses. Statistical analyses were performed with Minitab version 15.

9.3 Results

9.3.1 Patient characteristics during follow-up stratified by treatment with a RAAS inhibitor or oral glucocorticoid.

Of the 453 patients completed the follow-up visit, 378 were taking a RAAS inhibitor or oral glucocorticoid therapy and 75 were not taking a RAAS inhibitor or oral glucocorticoid therapy after discharge (Table 9-1).

Patients not taking a RAAS inhibitor or oral glucocorticoid treatment were older, more often female and more likely to have higher SBP and LVEF and less likely to have a history of angina compared with patients not taking the above agents or patients of the overall post-discharge cohort. Patients in the former group were also more likely to have lower potassium and eGFR and less likely to be treated with a beta-blocker at follow-up compared with patients in the other two groups.

Table 9-1. Characteristics of patients at follow-up stratified by treatment with a RAAS inhibitor* or oral glucocorticoid therapy.

Variable	Patients not taking a RAAS inhibitor or oral glucocorticoid therapy (n=75)	Patients taking a RAAS inhibitor or oral glucocorticoid therapy (n=378)	Overall cohort – follow-up (n=453)	p-value¶	p-value†
Age (years)	75 (68 - 82)	71 (65 - 77)	72 (66 - 78)	**0.006**	**0.015**
Female gender	42 (56)	139 (36.77)	181 (40)	**0.002**	**0.012**
NYHA class					
I	3 (4)	9 (2.38)	12 (2.6)	0.425	0.528
II	49 (65.33)	239 (63.23)	288 (63.6)	0.729	0.710
III	23 (30.67)	126 (33.33)	149 (32.9)	0.653	0.651
IV	0 (0)	4 (1.06)	4 (0.9)	¥	¥
Medical history					
HF	28 (37.33)	160 (42.33)	188 (41.5)	0.423	0.444
MI	30 (40)	165 (43.65)	195 (43)	0.560	0.713
Angina	32 (42.67)	216 (57.14)	248 (54.8)	**0.021**	**0.041**
Diabetes mellitus	16 (21.33)	127 (33.6)	143 (31.6)	**0.037**	0.064
Hypertension	56 (74.67)	240 (63.49)	296 (65.3)	0.063	0.098
AF	45 (60)	195 (51.59)	240 (53)	0.182	0.313
CVA/TIA	15 (20)	76 (20.11)	91 (20.1)	0.983	0.944
Physiological measurements					
BMI (kg/m^2)	26.37 (23.51 - 32.26)	27.67 (23.91 - 32.72)	27.6 (23.8 - 32.6)	0.413	0.559

Variable	Patients not taking a RAAS inhibitor or oral glucocorticoid therapy (n=75)	Patients taking a RAAS inhibitor or oral glucocorticoid therapy (n=378)	Overall cohort – follow-up (n=453)	p-value¶	p-value†
Weight (kg)	70.3 (57 - 132)	75.1 (23.9 - 90)	75 (62 - 89)	0.062	0.115
Pulse rate (bpm)	79 (66 - 91)	74 (65 - 86)	74 (65 - 86)	0.210	0.221
SBP (mmHg)	138 (123 - 150)	126.5 (112 - 142.25)	129 (114 - 144)	**<0.001**	**0.003**
DBP (mmHg)	70 (58 - 80)	66 (58 - 76)	67 (58 - 76)	0.184	0.245
Signs of fluid congestion					
Elevated JVP	7 (0.3)	53 (16.5)	60 (15.4)	0.197	0.364
Peripheral oedema	30 (39.5)	123 (32.6)	153 (33.8)	0.250	0.468
ECG rhythm					
SR	43 (57.33)	226 (59.79)	269 (59.4)	0.693	0.807
AF	28(37.33)	137 (36.24)	165 (36.4)	0.858	0.944
Echocardiography measurements					
LVEF (%)	45.5 (35.75 - 55)	40 (30 - 46)	40 (31 - 48)	**<0.001**	**0.001**
Laboratory measurements (blood)					
BNP (pg/ml)	471 (215 – 795)	387 (201.8 - 817)	396 (206 - 813)	0.441	0.532
Troponin I ≥ 0.04 (µg/L)	15 (20)	67 (17.72)	82 (18.1)	0.640	0.733
Sodium (mmol/L)	139 (138 - 141)	139 (137 - 141)	139 (137 - 141)	0.760	0.810
Potassium (mmol/L)	3.9 (3.6 - 4.1)	4.1 (3.8 - 4.4)	4.1 (3.8 - 4.3)	**0.001**	**0.005**
Urea (mmol/L)	9.8 (6.8 - 12)	8.5 (6.4 - 11.9)	8.6 (6.5 - 11.9)	0.535	0.637
Creatinine (µmol/L)	112 (89 - 151)	105 (87 -128)	106 (97 - 130.5)	0.156	0.205

Variable	Patients not taking a RAAS inhibitor or oral glucocorticoid therapy (n=75)	Patients taking a RAAS inhibitor or oral glucocorticoid therapy (n=378)	Overall cohort – follow-up (n=453)	p-value¶	p-value†
eGFR (ml/min/1.73m^2)	51 (34 - 60)	60 (44 - 60)	59 (43 - 60)	**0.005**	**0.015**
eGFR <60ml/min/1.73m^2	46 (61.33)	184 (48.68)	230 (50.8)	**0.045**	0.074
Cholesterol (total) (mmol/L)	4.0 (3.6 - 5.35)	4.05 (3.3 - 4.9)	4.0 (3.3 - 4.9)	0.257	0.374
HDL (mmol/L)	1.1 (1.0 - 1.4)	1.0 (0.8 - 1.3)	1.1 (0.8 -1.3)	**0.008**	**0.021**
CRP (mg/L)	6.2 (3.4 -20)	5.1 (2.5 - 11)	5.2 (2.6 - 12)	**0.034**	0.076
TSH (mIU/L)	1.6 (0.89 - 3.03)	1.5 (0.88 - 2.3)	1.5 (0.9 - 2.4)	0.468	0.539
Haemoglobin (g/dl)	12 (11.1 - 13.4)	12.6 (11.2 - 13.7)	12.5 (11.2 - 13.6)	0.233	0.376
Cardiovascular medication					
Diuretic	74 (98.67)	371 (98.15)	445 (98.2)	0.755	0.779
Beta-blocker	41 (54.67)	268 (70.9)	309 (68.2)	**0.006**	**0.027**
Digoxin	17 (22.67)	98 (25.93)	115 (25.4)	0.554	0.574
Anti-arrhythmic	7 (9.33)	19 (5.03)	26 (5.7)	0.143	0.247
Aspirin	43 (57.33)	210 (55.56)	253 (55.9)	0.777	0.740
Statin	54 (72)	281 (74.34)	335 (74)	0.673	0.772

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).
* ACE inhibitor/ARB or aldosterone blocker
¶Patients not taking a RAAS inhibitor or oral glucocorticoid (n=75) vs patients taking a RAAS inhibitor or oral glucocorticoid (n=378), Mann-Whitney test for continuous and χ^2 test for categorical variables.
† Patients not taking a RAAS inhibitor or oral glucocorticoid (n=75) vs patients of the overall post-discharge cohort (n=453), Mann-Whitney test for continuous and χ^2 test for categorical variables.
¥ Chi-Square approximation probably invalid

9.3.2 Levels of glucocorticoids during follow-up

Levels of plasma 11-deoxycortisol and cortisol, and the 11-deoxycortisol to cortisol ratio, during follow-up are presented below.

9.3.3 11-deoxycortisol

An 11-deoxycortisol level measured during follow-up was available in 68 of the 76 patients. The median (IQR) 11-deoxycortisol was 468.7 (305.4 – 739.3) pmol/L (Figure 9-1) and the mean (SD) 11-deoxycortisol was 589.7 (475.3) pmol/L. A frequency distribution histogram of 11-deoxycortisol levels in these patients is displayed in Figure 9-2. The minimum 11-deoxycortisol concentration was 14.0 pmol/L and the maximum 11-deoxycortisol concentration was 3051.5 pmol/L. Almost all patients (98.5%) had 11-deoxycortisol levels within the normal range (0 - 2017 pmol/L).

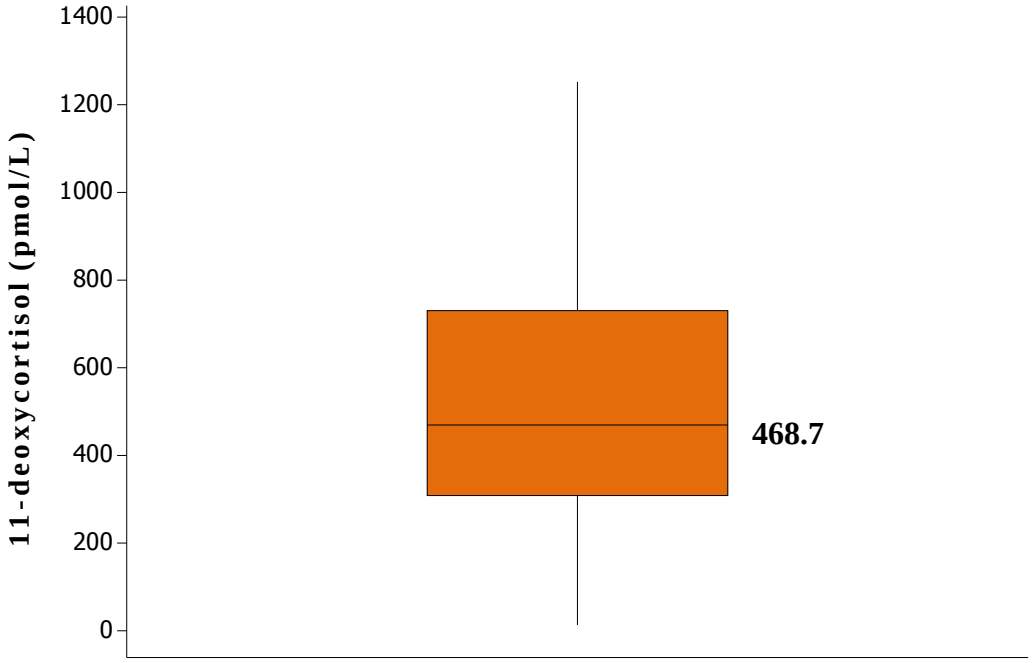


Figure 9-1. Box and whisker plot of 11-deoxycortisol concentrations showing the 2.5, 25, 50, 75 and 97.5 centiles

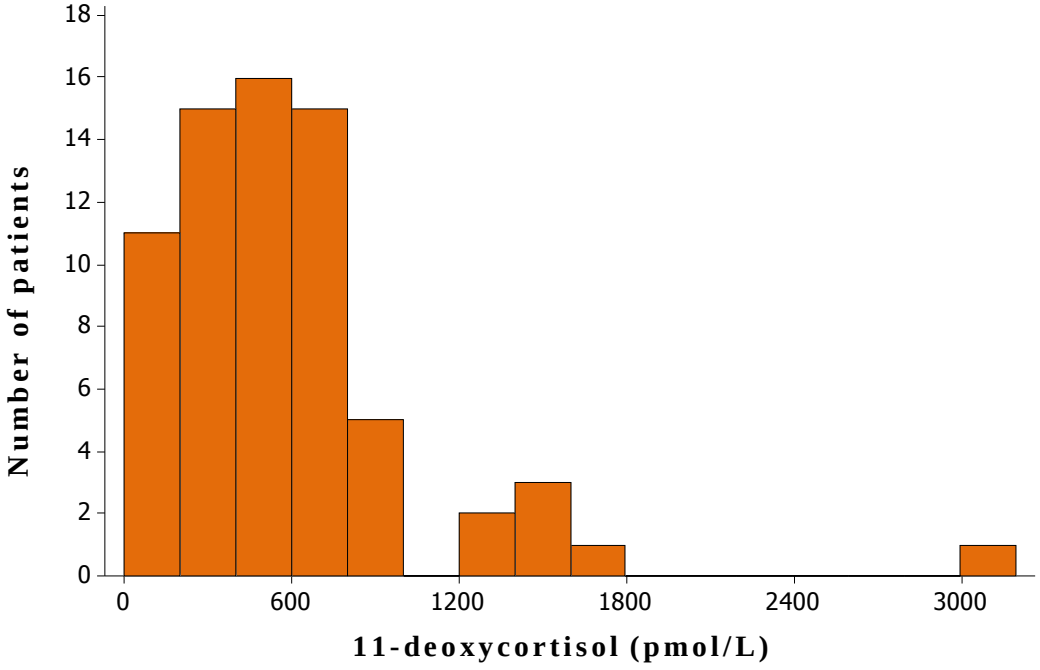


Figure 9-2. Frequency distribution histogram of 11-deoxycortisol concentrations

9.3.3.1 Cortisol

A cortisol level measured during follow-up was available in 73 of the 76 patients. The median (IQR) cortisol was 223.3 (153.7 - 310.4) nmol/L (Figure 9-3) and the mean (SD) cortisol was 262.4 (185.1) nmol/L. A frequency distribution histogram of cortisol levels in these patients is displayed in Figure 9-4. The minimum cortisol concentration was 12.1 nmol/L and the maximum cortisol concentration was 1166.4 nmol/L. The majority of patients (97.5%) had cortisol levels within the normal range (0 – 823nmol/L).

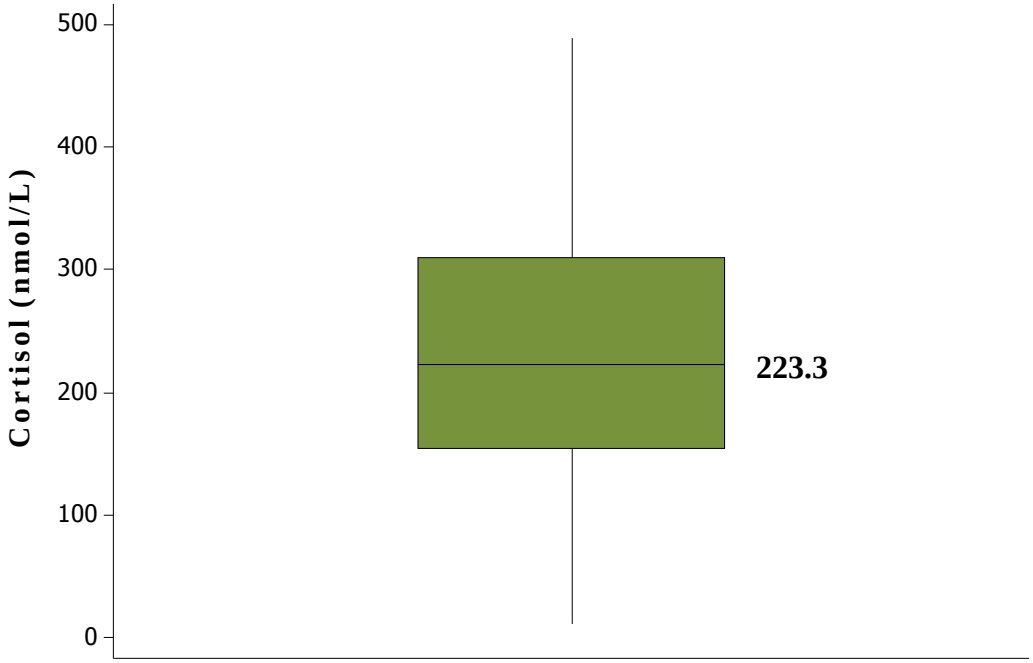


Figure 9-3. Box and whisker plot of cortisol concentrations showing the 2.5, 25, 50, 75 and 97.5 centiles

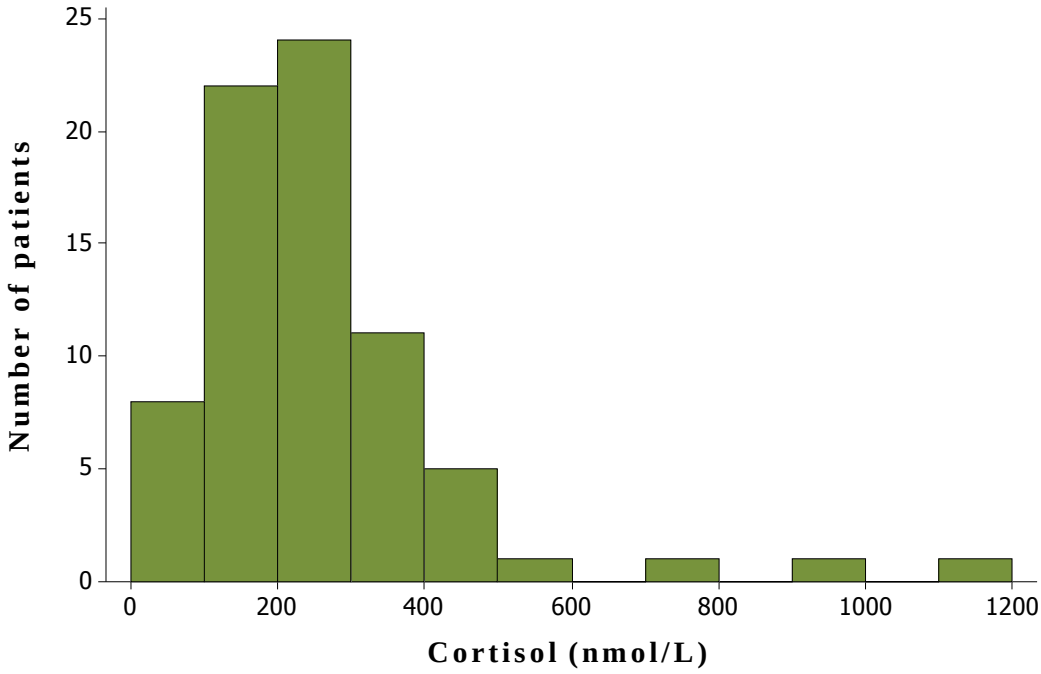


Figure 9-4. Frequency distribution histogram of cortisol concentrations

9.3.3.2 11-deoxycortisol to cortisol ratio

An 11-deoxycortisol to cortisol ratio could be calculated for 68 of the 76 patients studied. The median (IQR) 11-deoxycortisol to cortisol ratio was 2.06 (1.32 - 3.45) $x10^{-3}$ (Figure 9-5) and the mean (SD) 11-deoxycortisol to cortisol ratio was 2.70 (2.17) $x10^{-3}$. A frequency distribution histogram of 11-deoxycortisol to cortisol ratio in these patients is displayed in Figure 9-6. The minimum 11-deoxycortisol to cortisol ratio was 0.07 $x10^{-3}$ and the maximum 11-deoxycortisol to cortisol ratio was 13.5 $x10^{-3}$.

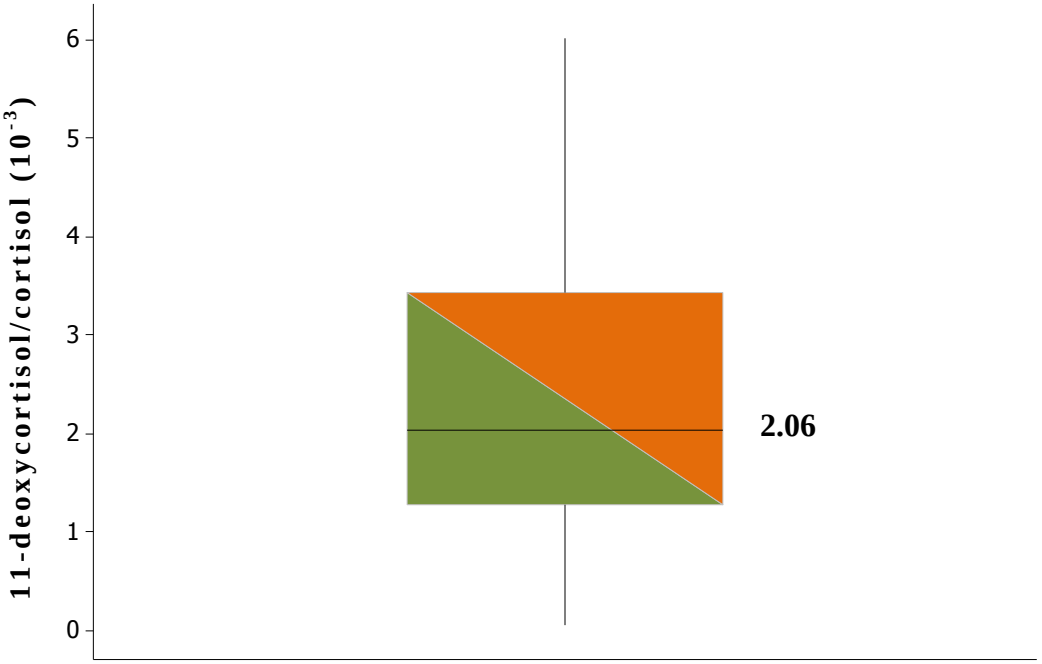


Figure 9-5. Box and whisker plot of 11-deoxycortisol/cortisol showing the 2.5, 25, 50, 75 and 97.5 centiles

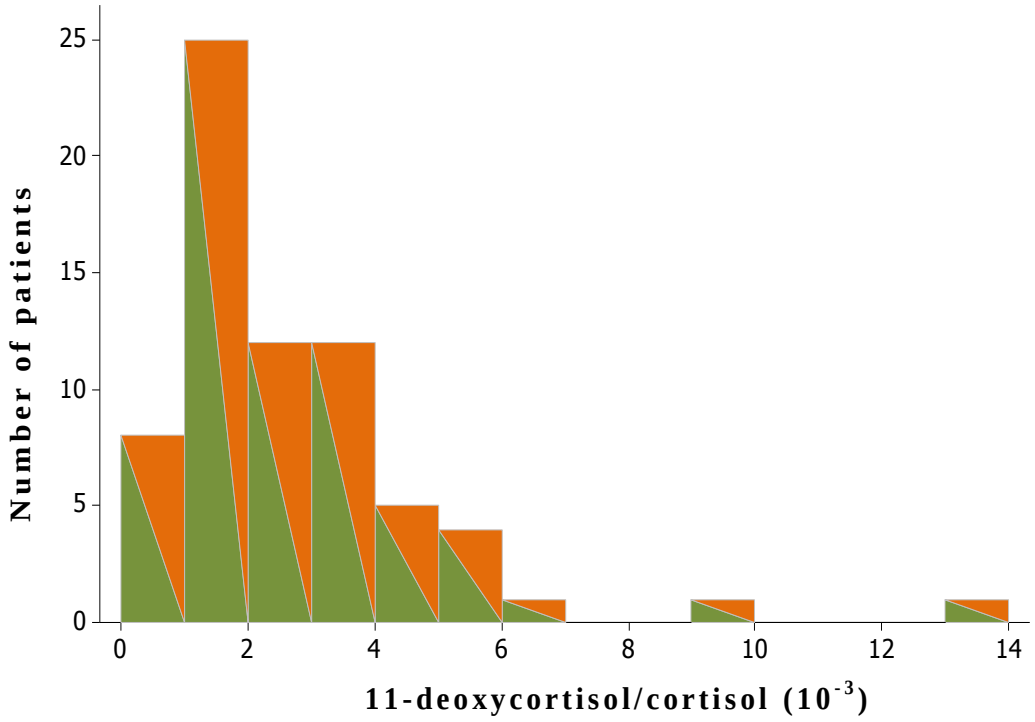


Figure 9-6. Frequency distribution histogram of 11-deoxycortisol to cortisol ratio

9.3.4 Patient characteristics according to glucocorticoid levels

The characteristics of patients not receiving a RAAS inhibitor or oral glucocorticoid therapy during follow-up were stratified according to the levels of 11-deoxycortisol and cortisol and the 11-deoxycortisol to cortisol ratio.

9.3.4.1 Patient characteristics according to 11-deoxycortisol levels

The cohort of 68 patients with measured 11-deoxycortisol levels was divided into two groups according to the median 11-deoxycortisol; patients with 11-deoxycortisol <468.7 pmol/L and patients with 11-deoxycortisol levels ≥ 468.7 pmol/L (Table 9-2).

Patients with lower 11-deoxycortisol levels were more likely to have lower cortisol and 11-deoxycortisol to cortisol ratio compared to patients with higher 11-deoxycortisol levels. In addition, a trend for higher PRC and aldosterone and lower SBP and eGFR was evident in these patients.

Table 9-2. Characteristics of patients not taking a RAAS inhibitor* or oral glucocorticoid therapy according to the median 11-deoxycortisol

Variable	11-deoxycortisol < 468.7 pmol/L (n=34)	11-deoxycortisol ≥ 468.7 pmol/L (n=34)	p-value†
Age (years)	75.5 (69.75 - 83.25)	75 (67.75 - 80)	0.280
Female gender	19 (55.88)	18 (52.94)	0.808
NYHA class			
I	2 (5.88)	1 (2.94)	0.555
II	24 (70.59)	21 (61.76)	0.442
III	8 (23.53)	12 (35.29)	0.287
Medical history			
HF	13 (38.24)	12 (35.29)	0.801
MI	13 (38.24)	15 (44.12)	0.622
Angina	15 (44.12)	15 (44.12)	1.000
Diabetes mellitus	4 (11.76)	10 (29.41)	0.072
Hypertension	25 (73.53)	25 (73.53)	1.000
AF	18 (52.94)	23 (67.65)	0.215
CVA/TIA	9 (26.47)	5 (14.71)	0.230
Physiological measurements			
BMI (kg/m^2)	26.36 (23.38 - 32.73)	26.34 (24.21 - 32.28)	0.835
Pulse rate (bpm)	77 (63.75 - 90.0)	74 (67 - 94)	0.628
SBP (mmHg)	134 (123.75 – 145.5)	140 (123 – 154.25)	0.310
DBP (mmHg)	69 (53.75 - 74.5)	71.5 (62.75 - 82)	0.170
Signs of fluid congestion			
Elevated JVP	2 (6.5)	3 (10)	0.614
Peripheral oedema	14 (41.2)	13 (37.1)	0.731
ECG rhythm			
SR	23 (67.65)	17 (50.0)	0.139
AF	10 (29.41)	14 (41.18)	0.310
Echocardiography measurements			
LVEF	45.5 (38.75 - 53.5)	46 (33 – 55.25)	0.797
LVEF <45%	16 (47)	14 (40)	0.478
Laboratory measurements (blood)			
BNP (pg/ml)	442.5 (213 – 858)	526.5 (231 - 787)	0.677
Troponin I ≥ 0.04 (µg/L)	5 (14.71)	9 (26.47)	0.230
Sodium (mmol/L)	138.5 (137 - 141)	140 (138 - 141)	0.187
Potassium (mmol/L)	3.8 (3.5 - 4.1)	3.95 (3.78 - 4.13)	0.119

Variable	11-deoxycortisol < 468.7 pmol/L (n=34)	11-deoxycortisol ≥ 468.7 pmol/L (n=34)	p-value†
Urea (mmol/L)	9.35 (6.95 - 10.7)	9.8 (6.15 - 13.03)	0.893
Creatinine (µmol/L)	115 (92 - 141.25)	111.5 (84.25 – 153.5)	0.650
eGFR (ml/min/1.73m^2)	50 (34 - 60)	56.5 (34 - 60)	0.440
eGFR <60ml/min/1.73m^2	24 (70.59)	18 (52.94)	0.134
Cholesterol (total) (mmol/L)	3.9 (3.38 - 4.8)	4.0 (3.58 - 5.53)	0.521
HDL (mmol/L)	1.3 (1.0 - 1.4)	1.05 (0.8 - 1.33)	0.070
CRP (mg/L)	5.65 (3.05 - 25.25)	5.85 (3.4 - 17.0)	0.813
TSH (mIU/L)	1.5 (0.75 - 2.85)	1.6 (0.89 - 3.03)	0.598
Cortisol (nmol/L)	200.4 (131.7 - 263.6)	291.2 (196 - 389.9)	**0.006**
11-deoxycortisol/cortisol (10^{-3})	1.47 (0.99 - 2.38)	3.07 (1.64 - 4.68)	**<0.001**
Aldosterone (pmol/L)	195.3 (106.8 - 342.2)	172.4 (87.7 - 319.8)	0.615
PRC (mIU/L)	47.45 (17 - 109.2)	33.30 (18.1 - 104.1)	0.659
Aldosterone/PRC	4.17 (1.94 - 9.62)	3.74 (1.83 - 12.63)	0.912
Haemoglobin (g/dl)	11.7 (11.3 - 13.3)	12.85 (11.3 - 13.7)	0.202
Cardiovascular medication			
Diuretic	33 (97.06)	34 (100)	- ¥
Beta-blocker	19 (55.88)	20 (58.82)	0.806
Digoxin	4 (11.76)	10 (29.41)	0.072
Anti-arrhythmic	4 (11.76)	2 (5.88)	0.393
Aspirin	21 (61.76)	18 (52.94)	0.462
Statin	26 (76.47)	26 (76.47)	1.000

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)

* ACE inhibitor/ARB or aldosterone blocker.

† Mann-Whitney test was used for continuous variables and χ^2 test for categorical variables.

¥ Chi-Square approximation probably invalid.

9.3.4.2 Patient characteristics according to cortisol levels

The cohort of 73 patients with measured cortisol levels was divided into two groups according to the median cortisol; patients with cortisol <223.3 pmol/L and patients with cortisol levels ≥ 223.3 pmol/L (Table 9-3).

Patients with higher cortisol levels were more likely to have higher 11-deoxycortisol, aldosterone, PRC and urea and lower 11-deoxycortisol to cortisol ratio. There was also a trend for higher BNP elevated troponin and lower eGFR in these patients. Patients with higher cortisol levels were more often in NHYA class III and less often in NYHA class II compared to patients with lower cortisol levels. Beta-blockers were more often prescribed in patients of the former group compared to patients of the latter group.

Table 9-3. Characteristics of patients not taking a RAAS inhibitor* or oral glucocorticoid therapy according to the median cortisol

Variable	Cortisol < 223.3 nmol/L (n=37)	Cortisol ≥ 223.3 nmol/L (n=36)	p-value†
Age (years)	75 (67.5 – 82)	75.5 (69 – 82.5)	0.551
Female gender	19 (51.35)	23 (63.89)	0.279
NYHA class			
I	2 (5.41)	1 (2.78)	0.572
II	28 (75.68)	22 (58.33)	0.115
III	7 (18.92)	14 (38.89)	0.060
Medical history			
HF	14 (37.84)	13 (36.11)	0.879
MI	15 (40.54)	13 (36.11)	0.697
Angina	15 (40.54)	16 (44.44)	0.736
Diabetes mellitus	5 (13.51)	10 (27.78)	0.132
Hypertension	25 (67.57)	29 (80.56)	0.206
AF	25 (67.57)	20 (55.56)	0.291
CVA/TIA	10 (27.03)	4 (11.11)	0.084
Physiological measurements			
BMI (kg/m^2)	26.52 (24.0 – 31.9)	26.11 (22.91 – 33.42)	0.860
Pulse rate (bpm)	79 (61.5 – 90)	74 (68 – 91.75)	0.540
SBP (mmHg)	140 (126.5 – 152)	134.5 (120 – 147)	0.270
DBP (mmHg)	73 (61.5 – 81)	67 (54.25 – 75.5)	0.229
Signs of fluid congestion			
Elevated JVP	3 (9.1)	3 (9.1)	1.000
Peripheral oedema	10 (27)	19 (51.4)	**0.032**
ECG rhythm			
SR	23 (62.16)	20 (52.78)	0.417
AF	12 (32.43)	15 (41.67)	0.414
Echocardiography measurements			
LVEF	46 (39.25 – 55)	46 (33.75 – 54.5)	0.991
LVEF <45%	20 (55.6)	21 (56.8)	0.918
Laboratory measurements (blood)			
BNP (pg/ml)	340 (193.5 – 700)	536 (298 – 1038)	0.105
Troponin I ≥ 0.04 (µg/L)	5 (13.51)	9 (25)	0.213
Sodium (mmol/L)	139 (137 – 141)	140 (138 – 141)	0.360
Potassium (mmol/L)	3.9 (3.6 – 4.15)	3.9 (3.6 – 4.1)	0.882

Variable	Cortisol < 223.3 nmol/L (n=37)	Cortisol ≥ 223.3 nmol/L (n=36)	p-value†
Urea (mmol/L)	8.1 (5.9 – 10.3)	10.4 (7.25 – 12.45)	**0.029**
Creatinine (µmol/L)	102 (88 – 125.5)	112.5 (92 – 153)	0.265
eGFR (ml/min/1.73m^2)	55 (39 – 60)	43 (33.25 – 60)	0.111
eGFR <60ml/min/1.73m^2	20 (54.05)	25 (69.44)	0.176
Cholesterol (total) (mmol/L)	3.9 (3.6 – 5.35)	4.05 (3.43 – 505)	0.683
HDL (mmol/L)	1.3 (1.0 – 1.4)	1.0 (0.9 – 1.4)	0.260
CRP (mg/L)	5.8 (3.3 – 26.0)	6.8 (3.9 – 17.0)	0.932
TSH (mIU/L)	1.5 (0.91 – 3.2)	1.7 (0.66 – 2.6)	0.783
11-deoxycortisol (pmol/L)	422.7 (200.8 – 634.4)	625.3 (337.4 – 943.6)	**0.015**
11-deoxycortisol/cortisol (10^{-3})	3.05 (1.63 – 4.04)	1.59 (1.19 – 2.62)	**0.004**
Aldosterone (pmol/L)	128.2 (81.5 – 257.6)	224.8 (128.6 – 363.1)	**0.025**
PRC (mIU/L)	33.8 (13.1 – 52.9)	61.9 (24.3 – 125.4)	**0.016**
Aldosterone/PRC	4.34 (1.82 – 10.48)	3.62 (1.99 – 9.86)	0.753
Haemoglobin (g/dl)	12.7 (11.5 – 13.4)	11.8 (10.73 – 13.08)	0.144
Cardiovascular medication			
Diuretic	36 (97.3)	36 (100)	- ¥
Beta-blocker	16 (43.24)	24 (66.67)	**0.044**
Digoxin	7 (18.92)	10 (27.78)	0.371
Anti-arrhythmic	3 (8.11)	4 (11.11)	0.663
Aspirin	20 (54.05)	21 (58.33)	0.713
Statin	26 (70.27)	29 (77.78)	0.465

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)

*ACE inhibitor/ARB or aldosterone blocker.

† Mann-Whitney test was used for continuous variables and χ^2 test for categorical variables.

¥ Chi-Square approximation probably invalid.

9.3.4.3 Patient characteristics according to 11-deoxycortisol to cortisol ratio

The cohort of 68 patients with calculated 11-deoxycortisol to cortisol ratio was divided into two groups according to the median 11-deoxycortisol to cortisol ratio; patients with 11-deoxycortisol to cortisol < 2.06 $x10^{-3}$ and patients with 11-deoxycortisol levels ≥ 2.06 $x10^{-3}$ (Table 9-4).

Patients with lower 11-deoxycortisol to cortisol ratio were more likely to have lower 11-deoxycortisol, SBP and haemoglobin and higher BNP and cortisol compared to patients with higher 11-deoxycortisol to cortisol ratio. There was also a trend for higher PRC and aldosterone, urea and CRP and elevated troponin in these patients. Patients with lower higher 11-deoxycortisol to cortisol ratio cortisol were also more often in NYHA class III compared to patients with higher 11-deoxycortisol to cortisol ratio.

Table 9-4. Characteristics of patients not taking a RAAS inhibitor* or oral glucocorticoid therapy according to the median 11-deoxycortisol to cortisol

Variable	11-deoxycortisol/ cortisol < 2.06 (n=34)	11-deoxycortisol/ cortisol ≥ 2.06 (n=34)	p-value†
Age (years)	75.50 (72 – 83.25)	74.50 (67.75 – 80)	0.234
Female gender	21 (61.76)	16 (47.06)	0.223
NYHA class			
I	0 (0)	3 (8.82)	0.076
II	20 (58.82)	25 (73.53)	0.200
III	14 (41.18)	6 (17.65)	**0.033**
Medical history			
HF	12 (35.29)	13 (38.24)	0.801
MI	13 (34.24)	15 (44.12)	0.622
Angina	13 (38.24)	17 (50)	0.329
Diabetes mellitus	7 (20.59)	7 (20.59)	1.000
Hypertension	26 (76.47)	24 (70.59)	0.582
AF	17 (50)	24 (70.59)	0.083
CVA/TIA	6 (17.65)	8 (23.53)	0.549
Physiological measurements			
BMI (kg/m^2)	25.48 (22.86 – 32.75)	27.36 (24.41 – 32.37)	0.275
Pulse rate (bpm)	80 (67.75– 91.25)	73.5 (59.75 – 91)	0.585
SBP (mmHg)	131.5 (119.5 – 145.5)	143 (129 – 154.5)	**0.032**
DBP (mmHg)	69 (58 – 75.5)	73 (62.25 – 82)	0.377
Signs of fluid congestion			
Elevated JVP	2 (6.1)	3 (10.7)	0.509
Peripheral oedema	16 (45.7)	26 (76.5)	**0.009**
ECG rhythm			
SR	22 (64.71)	18 (52.94)	0.324
AF	10 (29.41)	14 (41.18)	0.310
Echocardiography measurements			
LVEF	43 (32.88 – 50.50)	47.5 (39.75 – 56)	0.119
LVEF <45%	17 (48.6)	22 (64.7)	0.176
Laboratory measurements (blood)			
BNP (pg/ml)	622.5 (289 – 1083)	436.5 (184.5 – 639.8)	**0.047**
Troponin I ≥ 0.04 (µg/L)	9 (26.47)	5 (14.71)	0.230
Sodium (mmol/L)	139 (138 – 141)	140 (137 – 141)	0.801

Variable	11-deoxycortisol/ cortisol < 2.06 (n=34)	11-deoxycortisol/ cortisol ≥ 2.06 (n=34)	p-value†
Potassium (mmol/L)	3.8 (3.5 – 4.1)	4.0 (3.7 – 4.2)	0.116
Urea (mmol/L)	10.05 (7.78 – 11.85)	7.35 (5.68 – 11.23)	0.092
Creatinine (µmol/L)	118 (92.75 – 153)	106 (81.75 – 143.5)	0.215
eGFR (ml/min/1.73m^2)	46 (33.75 – 60)	59 (37.75 – 60)	0.108
eGFR <60ml/min/1.73m^2	25 (73.53)	17 (50)	**0.046**
Cholesterol (total) (mmol/L)	3.9 (3.38 – 5.43)	3.95 (3.58 – 4.8)	0.793
HDL (mmol/L)	1.3 (1.0 – 1..65)	1.1 (0.9 – 1.3)	0.116
CRP (mg/L)	8.5 (3.85 – 25.5)	4.7 (3.3 – 15.0)	0.162
TSH (mIU/L)	1.7 (0.66 – 3.65)	1.45 (0.86 – 2.68)	0.707
Cortisol (nmol/L)	279 (208.4 – 395.4)	189.5 (116.4 – 280.8)	**<0.001**
11-deoxycortisol (pmol/L)	329.5 (240.6 – 494.8)	686.6 (454.9 – 893.8)	**<0.001**
Aldosterone (pmol/L)	208.4 (120.2 – 375.9)	142.1 (85.1 – 289.3)	0.166
PRC (mIU/L)	53.6 (20.9 – 131.8)	34.95 (12.2 – 93.3)	0.131
Aldosterone/PRC	3.71 (2.17 – 8.80)	4.19 (1.65 – 13.18)	0.980
Haemoglobin (g/dl)	11.7 (10.93 - 13.1)	12.5 (11.5 - 13.75)	**0.048**
Cardiovascular medication			
Diuretic	34 (100)	33 (97.06)	- ¥
Beta blocker	18 (52.94)	21 (61.76)	0.462
Digoxin	6 (17.65)	8 (23.53)	0.549
Anti-arrhythmic	5 (14.71)	1 (2.94)	0.087
Aspirin	20 (58.82)	19 (55.88)	0.806
Statin	22 (64.71)	30 (88.24)	**0.022**

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)

*ACE inhibitor/ARB or aldosterone blocker

† Mann-Whitney test was used for continuous variables and χ^2 test for categorical variables.

¥ Chi-Square approximation probably invalid

9.3.5 Levels of glucocorticoids during hospital admission and follow-up in patients not receiving a RAAS inhibitor or oral glucocorticoid therapy

Of the 75 patients not taking a RAAS inhibitor or oral glucocorticoid therapy during follow-up, 55 were not taking the above agents prior to hospital admission with decompensated HF. The demographic characteristics, medical history and LVEF in these patients are presented in Table 9-5.

Table 9-5. Demographics, medical history and echocardiographic measurements in patients not taking a RAAS inhibitor* or oral glucocorticoid therapy prior to admission and at follow-up (n=55) and in patients of the overall cohort during the hospital admission (n=722) and the follow-up visit (n=453)

Variable	Patients not taking a RAAS inhibitor or oral glucocorticoid therapy (n=55)	Overall cohort – hospital (n=722)	Overall cohort – follow-up (n=453)	p-value¶	p-value†
Age (years)	76 (69 - 82)	74 (68 - 81)	72 (66 - 78)	0.289	**0.007**
Female gender	31 (56.4)	332 (46)	181 (40)	0.137	**0.020**
Medical history					
HF	15 (27.3)	320 (44.3)	188 (41.5)	**0.014**	**0.042**
MI	21 (38.2)	322 (44.6)	195 (43)	0.356	0.491
Angina	26 (47.3)	396 (54.8)	248 (54.7)	0.277	0.294
Diabetes mellitus	9 (16.4)	227 (31.4)	143 (31.6)	**0.019**	**0.020**
Hypertension	40 (72.7)	478 (66.2)	296 (65.3)	0.323	0.274
AF	31 (56.4)	387 (53.6)	240 (53)	0.692	0.635
CVA/TIA	13 (23.6)	155 (21.5)	91 (20.1)	0.707	0.538
Echocardiographic measurements					
LVEF (%)	47 (37.5 - 55)	-	40 (31 - 48)	-	**0.001**
LVSD (Y)	22 (52.4)	341 (66.6)	-	0.062	-

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).
*ACE inhibitor/ARB or aldosterone blocker
¶ Patients not taking a RAAS inhibitor or an oral glucocorticoid prior to admission and during follow-up (n=55) vs overall hospitalised cohort (n=722), Mann-Whitney test for continuous and χ^2 test for categorical variables.
† Patients not taking a RAAS inhibitor or an oral glucocorticoid prior to admission and during follow-up (n=55) vs overall post-discharge cohort (n=453), Mann-Whitney test for continuous and χ^2 test for categorical variables.

Patients not taking a RAAS inhibitor or oral glucocorticoid treatment were older, more often female and were more likely to have higher LVEF compared with patients of the overall post-discharge cohort. This group was also less likely to have history of HF and diabetes compared with the overall post-discharge or hospitalised cohort.

The physiological and laboratory measurements of these patients during hospital admission and follow-up and the medication prior to admission and after discharge are presented in Table 9-6.

Table 9-6. Clinical characteristics, physiological and laboratory measurements and medication in patients not taking a RAAS inhibitor* or oral glucocorticoid therapy during hospital admission and follow-up (n=55)

Variable	During admission (n=55)	During follow-up (n=55)	p-value†
NYHA class			
I	0 (0)	2 (3.6)	- ¥
II	20 (36.4)	37 (67.3)	**0.001**
III	29 (52.7)	16 (29.1)	**0.026**
IV	6 (11)	0 (0)	- ¥
Physiological measurements			
BMI (kg/m^2)	28 (24.2 - 34.1)	26.5 (23 - 33.5)	**<0.001**
Weight (kg)	73.5 (57 - 90)	69.7 (56.4 - 87)	**<0.001**
Pulse rate (bpm)	86 (72 - 99)	79 (67 - 93)	**0.014**
SBP (mmHg)	140 (125 - 155)	142 (128 - 153)	0.572
DBP (mmHg)	80 (68 - 90)	70 (58 - 80)	**0.001**
Signs of fluid congestion			
Elevated JVP	39 (78)	4 (8.3)	**<0.001**
Peripheral oedema	36 (65.5)	21 (38.2)	**0.001**
Laboratory measurements (blood)			
BNP (pg/ml)	647 (256 - 1715)	457 (207 - 772)	**<0.001**
Sodium (mmol/L)	138 (136 - 141)	139 (137 - 141)	0.081
Potassium (mmol/L)	4.1 (3.8 - 4.6)	3.9 (3.6 - 4.1)	**0.001**
Urea (mmol/L)	8.8 (5.6 - 10.9)	9.2 (6.8 - 11)	0.175
Creatinine (µmol/L)	112 (75 - 144)	115 (89 - 151)	**0.002**
eGFR (ml/min/1.73m^2)	53 (35 - 60)	51 (30 - 60)	0.051
eGFR <60ml/min/1.73m^2	34 (62)	36 (65.5)	0.727
Cholesterol (total) (mmol/L)	3.9 (3.4 - 5.3)	4.1 (3.6 - 5.5)	0.211
HDL (mmol/L)	1.1 (0.9 - 1.4)	1.1 (1.0 - 1.4)	0.327
CRP (mg/L)	11 (4.6 - 37)	6.9 (3.9 - 21.3)	**0.001**
TSH (mIU/L)	2.3 (1.2 - 3.8)	1.5 (0.7 - 2.5)	0.344
Cortisol (nmol/L)	346.9 (259.7 - 460)	220.4 (151 - 297.3)	**<0.001**
11-deoxycortisol (pmol/L)	536 (323 - 1047)	423.8 (258.8 - 752.7)	0.433
11-deoxycortisol/cortisol (10^{-3})	1.68 (1.14 - 3.44)	2.04 (1.30 - 3.42)	0.266
Aldosterone (pmol/L)	118.7 (52.8 - 252.8)	185 (109.7 - 298.8)	0.111
PRC (mIU/L)	31.2 (9.0 - 67.3)	46.5 (18.5 - 107)	**0.014**
Aldosterone/PRC	3.28 (1.65 - 8.14)	3.85 (2.10 - 13.41)	0.283
Haemoglobin (g/dl)	12 (10.3 - 13.4)	12 (11 - 13.4)	0.374

Variable	During admission (n=55)	During follow-up (n=55)	p-value†
Cardiovascular medication			
Diuretic	30 (54.5)	54 (98.2)	**<0.001**
Beta-blocker	27 (49)	27 (49)	1
Digoxin	8 (14.5)	9 (16.4)	1
Anti-arrhythmic	5 (9)	5 (9)	1
Aspirin	31 (56)	32 (58.2)	1
Statin	33 (60)	39 (71)	0.146

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)

* ACE inhibitor/ARB or aldosterone blocker.

† Wilcoxon matched pairs test was used for continuous variables and McNemars's test was used for categorical variables.

¶ medication prior to hospital admission for patients studied during the hospital admission.

¥ Chi-Square approximation probably invalid**.**

Cortisol levels were lower at follow-up compared with the hospital admission. There was a trend for lower 11-deoxycortisol levels and higher 11-deoxycortisol to cortisol ratio at follow-up, but that did not reach statistical significance. Patients were taking diuretics more frequently and were more likely to have higher PRC and creatinine and lower weight, BNP and eGFR after discharge. The pulse rate, DBP, potassium and CRP were also lower at follow-up compared with hospital admission.

9.4 Discussion

9.4.1 Patient characteristics according to glucocorticoid secretion during follow-up

The levels of 11-deoxycortisol and cortisol and the 11-deoxycortisol to cortisol ratio in patients not taking a RAAS inhibitor or oral glucocorticoid therapy at follow-up were similar to the levels of above glucocorticoids and their ratio in the overall post-discharge cohort. Cortisol was associated with higher levels of RAAS mediators at the follow-up visit similar to the hospital admission. Apart from the associations with the RAAS mediators, cortisol levels were also higher in patients with higher urea levels. Glucocorticoids are normally excreted by the kidneys and chronic renal failure has been reported to result in prolonged half-life of cortisol (399). Urea is likely to reflect a decline in glomerular filtration rate due to renal hypoperfusion in these patients. Nevertheless, it has been suggested that in patients with HF, blood urea is raised not only due to the decline in glomerular filtration rate but also due to the increased reabsorption by the nephrons secondary to activation of RAAS and other neurohumoral pathways (400) (401). Under these circumstances, urea not only reflects the local reduction in renal perfusion but also the systemic hypoperfusion with activation of the RAAS and other compensatory cascades. Thus, the above pathophysiological pathways may explain the association between cortisol with higher urea levels at the follow-up visit. Alternatively, urea and cortisol may reflect a state of higher protein breakdown in these patients; cortisol exerts catabolic effects on protein metabolism (402), which in turn results in an increase of urea production. Thus, patients with higher urea and cortisol levels might represent a subgroup characterised by prominence of the catabolic processes.

Patients with lower 11-deoxycortisol to cortisol ratio had lower SBP and higher PRC and aldosterone. That indicates that chronic ACTH stimulation, as reflected by the up-regulation of the late enzymatic step in cortisol synthesis, is associated with greater RAAS activity in patients with stable HF similar to patients with decompensated HF. Correspondingly, these

patients had higher BNP, higher levels of inflammatory markers and worse kidney function reflecting the stimulation of glucocorticoid secretion in patients with worse HF. These associations were replicated in the overall post-discharge cohort (Table 13-12 in the Appendix) re-iterating the notion of greater HPA activity in patients with raised prognostic markers. The only exception was the absence of the higher RAAS activity in patients with lower 11-deoxcortisol to cortisol ratio; however, this may represent a confounding effect of RAAS inhibitors in the overall cohort.

In summary, most of the associations among glucocorticoid levels, HPA activity and markers of HF severity seen in patients with decompensated HF were replicated in patients with stable HF at the follow-up. These findings provide evidence that inhibition of 11beta-hydroxylase, as discussed in section 8.4.1, may be translated into clinical benefit in patients with chronic HF.

9.4.2 Change in glucocorticoid secretion from admission to follow-up in patients not taking an oral glucocorticoid or a RAAS inhibitor

Cortisol but not 11-deoxycortisol levels were lower at the follow-up visit compared with hospital admission in patients not taking oral glucocorticoid therapy or a RAAS inhibitor at both time points. Glucocorticoid levels were taken at different time periods during hospital admission and at the follow-up visit making the interpretation of these results not straightforward. Cortisol levels, as shown in section 5.3.4, were not different between hospital admission and follow-up in patients who had blood samples collected only in the morning at both time points. These findings argue against a potential impact of clinical improvement on the changes in cortisol levels. Thus, the lower levels of cortisol at follow-up in this study provide further evidence for a diurnal pattern in glucocorticoid secretion in patients with HF and in accordance with previous findings (section 5.3.4).

In summary, these findings indicate that glucocorticoid secretion in patients with HF maintains the characteristic circadian pattern observed in healthy subjects.

10. Prognostic value of RAAS mediators and glucocorticoid levels in patients with decompensated HF

10.1 Introduction

The prognostic importance of RAAS mediators has been extensively investigated in patients with chronic HF (55) (284) (285). Similarly, cortisol has been examined with respect to prognosis in patients with chronic HF (72) (73). Little is known about the prognostic significance of plasma levels of RAAS mediators and glucocorticoids in patients with decompensated HF (340). Moreover, their importance in patients with diastolic HF remains unclear. The aim of this chapter is to evaluate the prognostic value of RAAS mediators and plasma glucocorticoids in a cohort of patients with HFrSF and HFpSF during hospital admission.

10.2 Methods

The study design and laboratory measurements were described in sections 2.3.1 & 2.3.2. All patients enrolled in the study during hospital admission were included in the analyses irrespective of background therapy. The primary outcome in this study was all-cause mortality, defined as death in and out of the hospital from any cause. All-cause mortality was determined by the death certificates and the relevant information was linked to the study database through the ISD of the National Scottish Health Service. All study participants were linked with the ISD following consent and enrollment in the study. Survival was defined as the period from the enrollment in the study during hospital admission until the time of death or the censor date on 28th of August 2011.

For the outcome analyses, Kaplan-Meier event-free (time to death) survival curves were constructed for each of RAAS mediators and corticosteroids in the overall hospitalised cohort. The variables examined were PRC, aldosterone, 11-deoxycortisol, cortisol and the aldosterone to PRC ratio and 11-deoxycortisol to cortisol ratio. The log-rank test was used for

the comparison of the survival curves with each of these variables entered as quartiles or dichotomised according to the median (Q2) and the 75th percentile (Q3). Cox proportional hazard models were employed to calculate the hazard ratio (HR) and confidence interval (CI) of all-cause mortality over time with each variable entered as categorical (quartiles and dichotomised according to Q2 and Q3) and continuous (log-transformed). Firstly, univariate analyses were performed with each (neuro)hormone and their ratio as the only variable. All the variables found to be significantly associated with all-cause mortality on univariate analyses were included separately in multivariate Cox proportional hazards models. These models included a set of independent markers for all-cause mortality identified by backwards selection in a multivariate model with predetermined variables not including the variables in question; the prespecified variables were age, gender, previous hospitalisation with HF, history of COPD, SBP, LVSD, pulse rate, serum sodium and urea, eGFR, albumin, BNP, troponin and haemoglobin. The univariate and multivariate models were calculated for the full follow-up and up to 1 year from the hospital admission. All statistical analyses were performed using R version 2.15.1.

10.3 Results

10.3.1 Baseline characteristics – Follow-up

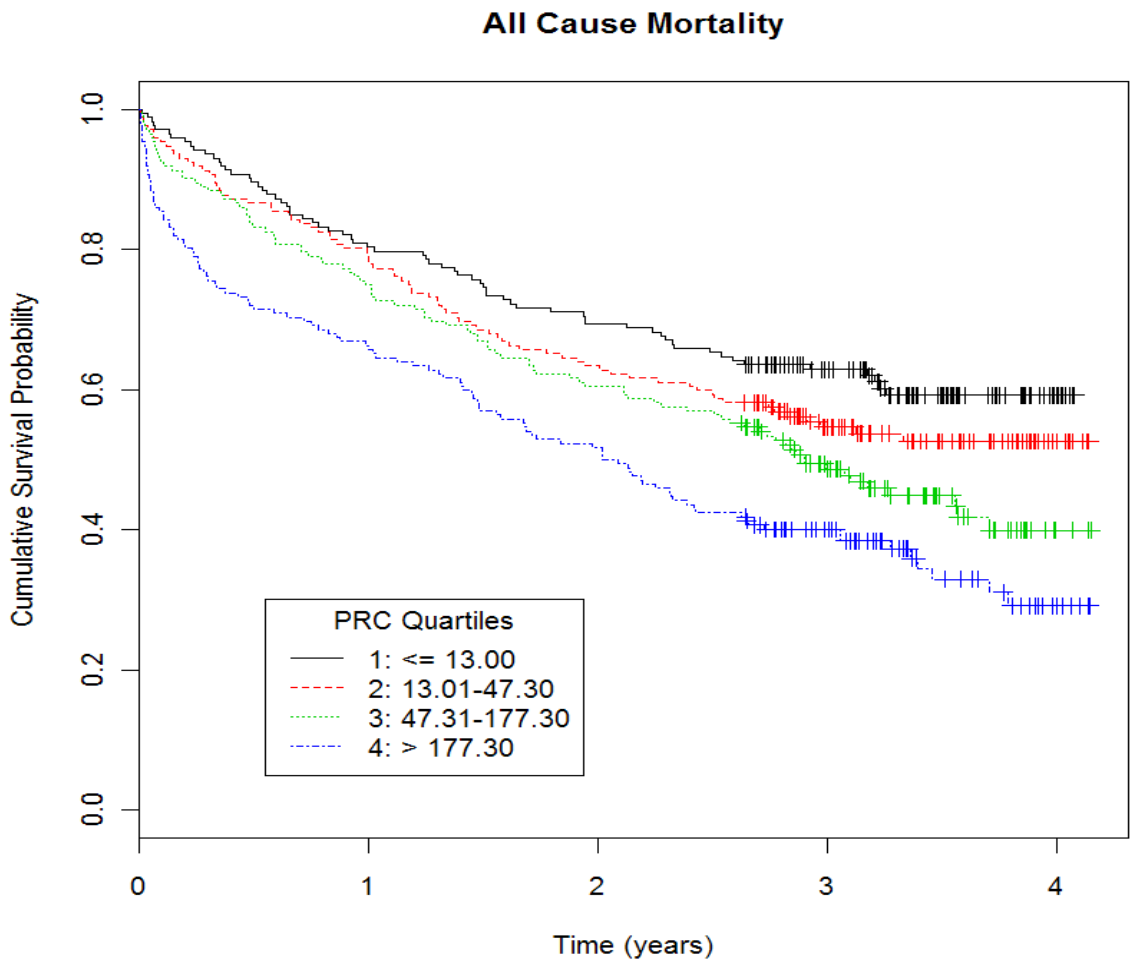
722 patients were included in this study. The patient characteristics were described in section 4.3.1. The median (IQR) follow-up was 998 (365 – 1217) days.

10.3.2 PRC and corticosteroid levels as univariate predictors of all-cause mortality

PRC and aldosterone concentrations, 11-deoxycortisol and cortisol levels and the aldosterone to PRC and 11-deoxycortisol to cortisol ratio were examined in relation to outcomes in the overall population. The association of each of these variables with all-cause mortality is presented below.

PRC

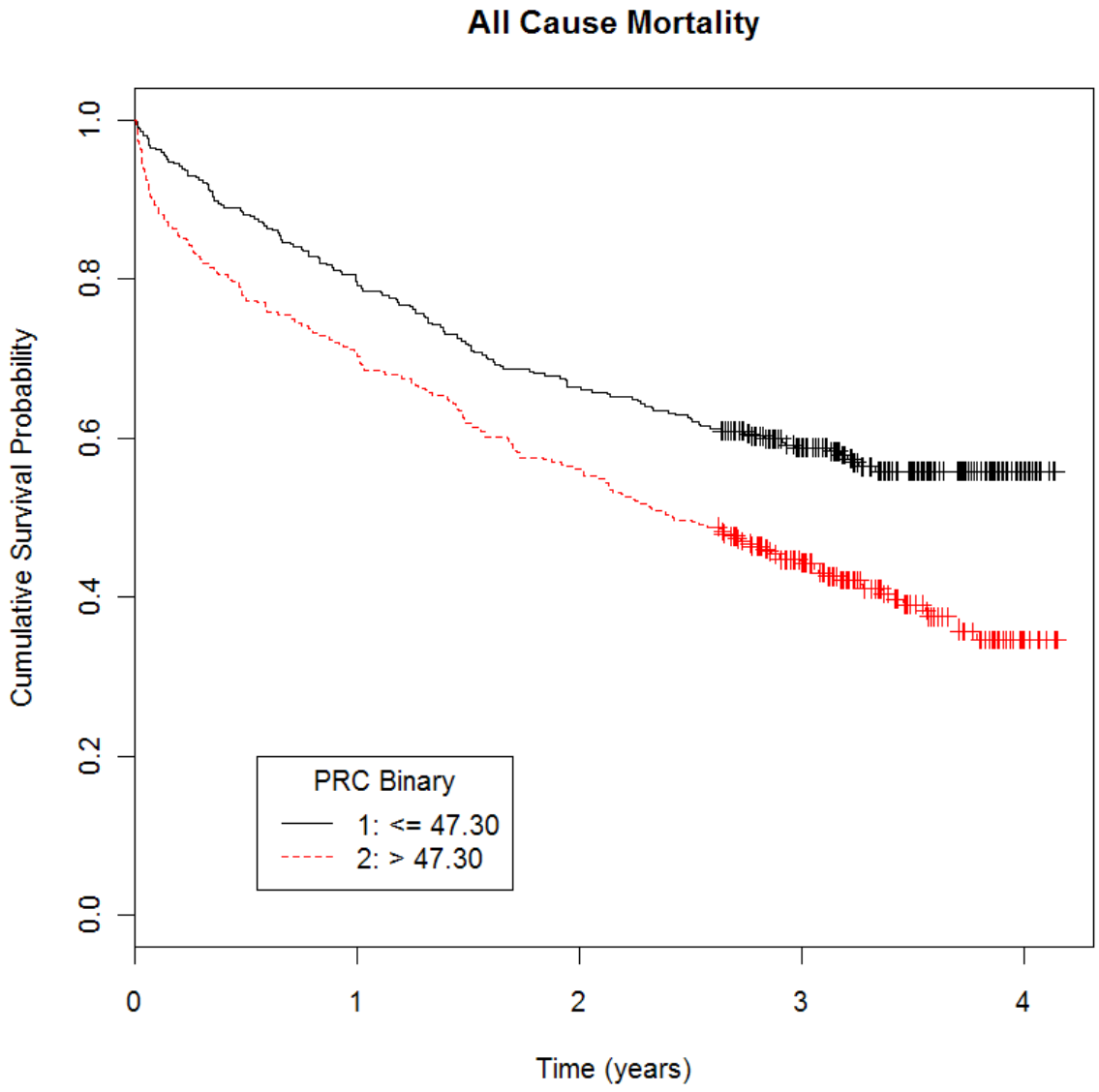
The Kaplan-Meier curves for all-cause mortality using PRC as quartiles are displayed in Figure 10-1.The rate of all-cause mortality increased across the quartiles of PRC. Patients with the higher PRC quartile had worse prognosis compared with the other groups (log-rank p-value < 0.001).



	No. at risk				
	Time = 0	1 year	2 year	3 year	4 year
PRC ≤ 13.00	173	139	120	83	10
PRC 13.01 – 47.30	172	135	109	68	9
PRC 47.31 – 177.30	172	129	104	64	3
PRC > 177.30	172	114	89	52	6

Figure 10-1. Kaplan–Meier event-free curves for patients with decompensated HF according to PRC quartiles

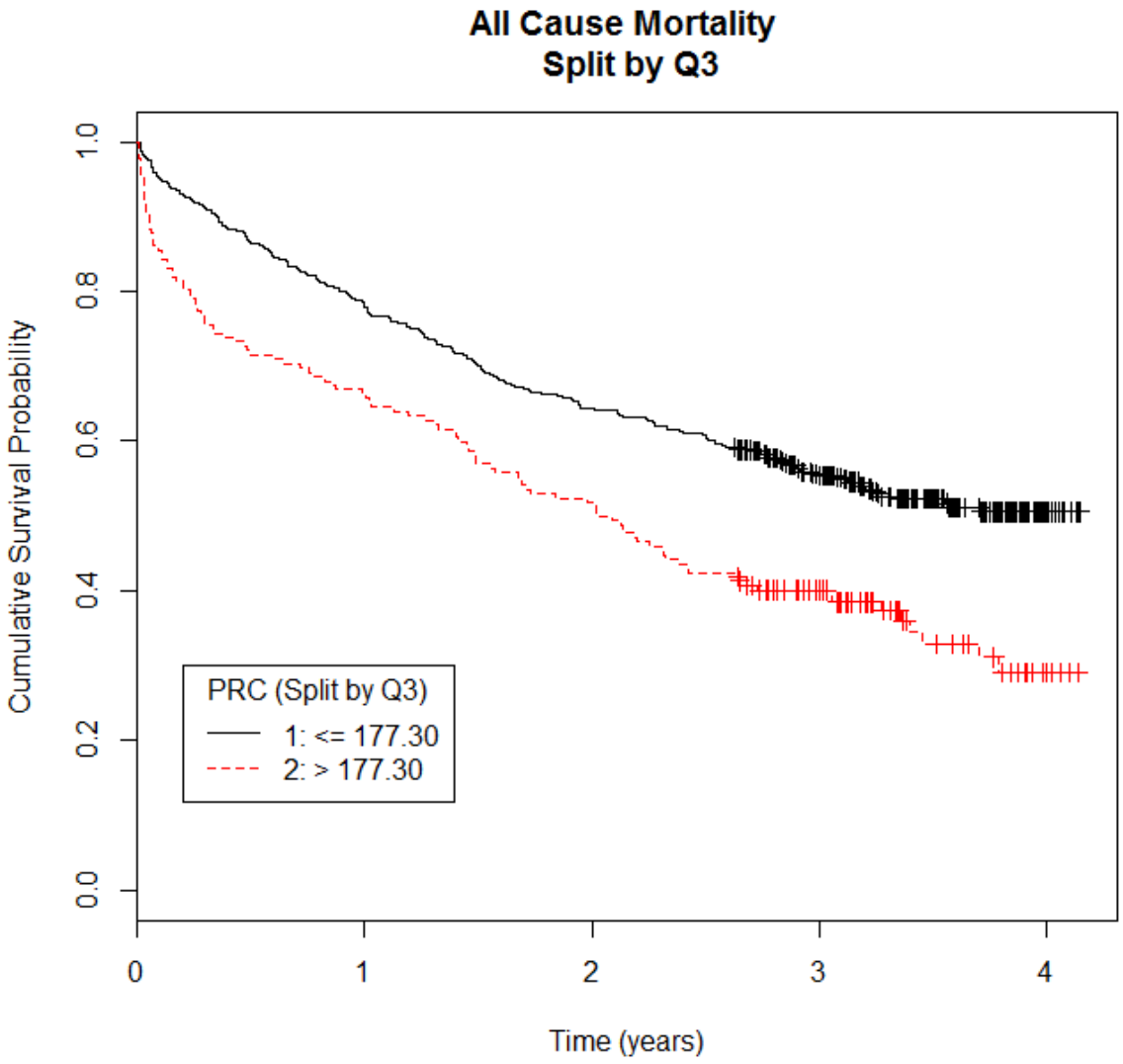
Likewise, patients with PRC above median had worse prognosis compared to patients with PRC below median (log-rank p-value < 0.001) (Figure 10-2 below).



	No. at risk				
	Time = 0	1 year	2 year	3 year	4 year
PRC ≤ 47.30	345	274	229	151	19
PRC > 47.30	344	243	193	116	9

Figure 10-2. Kaplan–Meier event-free curves for patients with decompensated HF according to median PRC

Similar results were produced when PRC was dichotomised according to the 75th percentile (log-rank p-value < 0.001) (Figure 10-3 below).



	No. at risk				
	Time = 0	1 year	2 year	3 year	4 year
PRC ≤ 177.3	517	403	333	215	22
PRC > 177.3	172	114	89	52	6

Figure 10-3. Kaplan–Meier event-free curves for patients with decompensated HF according to PRC 75th percentile

The univariate analyses of the Cox proportional hazard model for PRC as a predictor of all-cause mortality analysed as a categorical variable (quartiles or dichotomised by Q2 and Q3) or continuous variable (log-normalised) are presented in Table 10-1 below. PRC was positively associated with all-cause mortality with a HR of 0.474 (0.351, 0.642) for the lowest versus the highest PRC quartile and a HR of 0.620 (0.501, 0.766) for patients with PRC below median versus patients with PRC above median.

Table 10-1. Univariate Cox regression analysis of PRC at baseline for all-cause mortality with PRC entered as categorised (quartiles/dichotomised according to Q2 and Q3) and continuous (log-transformed variable).

Variable	HR (95% CI)	p-value
PRC (Quartiles) (mIU/L)		**<0.001**
≤ 13.00	**0.474 (0.351, 0.642)**	**<0.001**
13.01 – 47.30	**0.588 (0.441, 0.785)**	**<0.001**
47.31 – 177.30	**0.730 (0.554, 0.960)**	**0.0246**
> 177.30	1 (-)	
PRC (Split by Q2) (mIU/L)		**<0.001**
≤ 47.30	**0.620 (0.501, 0.766)**	
> 47.30	1 (-)	
PRC (Split by Q3) (mIU/L)		**<0.001**
≤ 177.30	**0.593 (0.574, 0.743**)	
> 177.30	1 (-)	
Log(PRC)	**1.104 (1.064, 1.145)**	**<0.001**

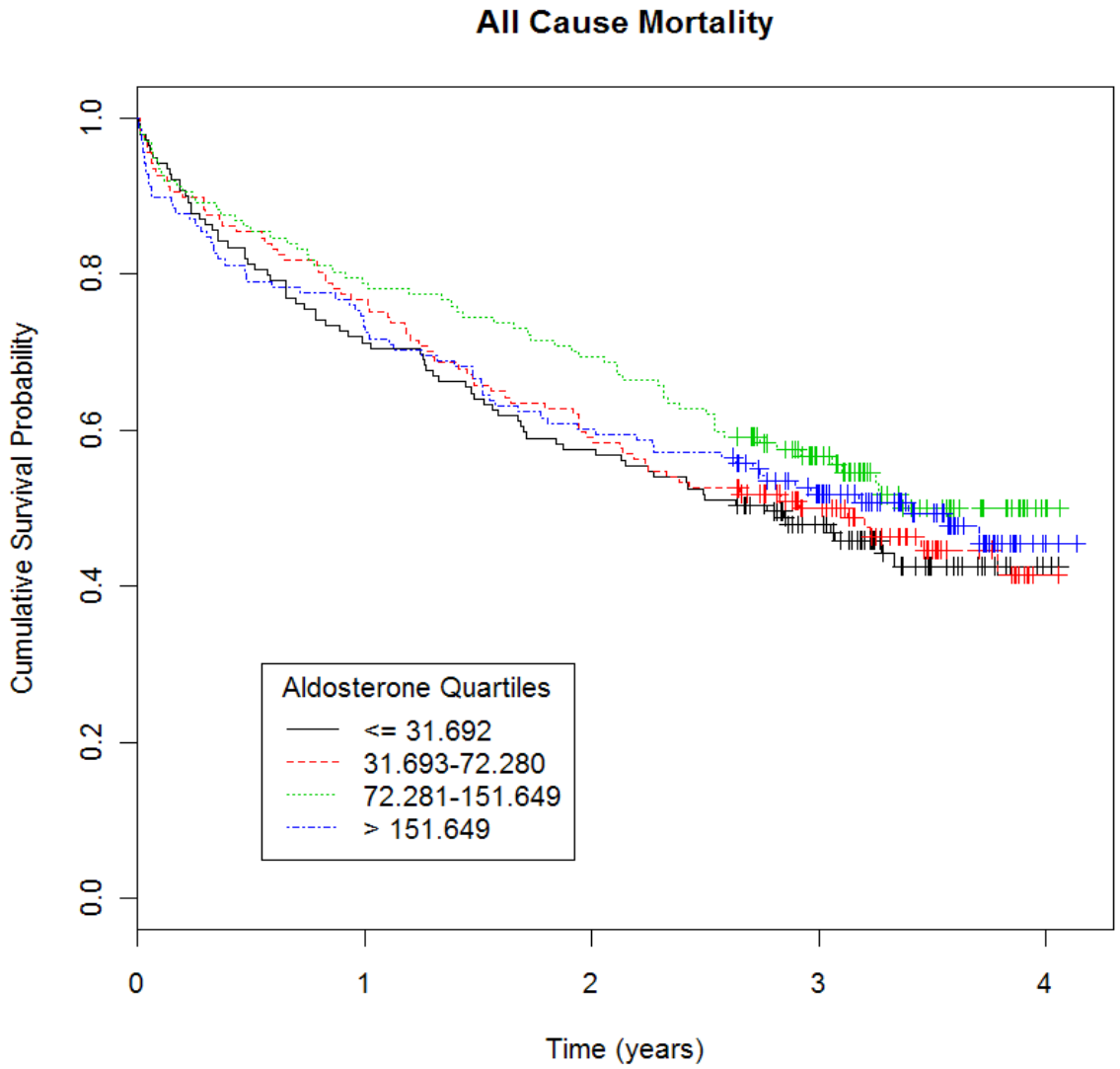
Univariate results for PRC as a predictor of all-cause mortality censoring the outcomes at 1 year after hospital admission is presented in Table 10-2.

Table 10-2. Univariate Cox regression analysis of PRC at baseline for all-cause mortality with PRC entered as categorised (quartiles/dichotomised according to Q2 and Q3) and continuous (log-transformed variable) censoring the outcomes at 1 year after hospital admission.

Variable	HR (95% CI)	p-value
PRC (Quartiles) (mIU/L)		**0.0038**
≤ 13.00	**0.497 (0.326, 0.760)**	**0.0012**
13.01 – 47.30	**0.556 (0.368, 0.840)**	**0.0053**
47.31 – 177.30	**0.663 (0.447, 0.984)**	**0.0411**
> 177.30	1 (-)	
PRC (Split by Q2) (mIU/L)		**0.0040**
≤ 47.30	**0.640 (0.473, 0.868)**	
> 47.30	1 (-)	
PRC (Split by Q3) (mIU/L)		**<0.001**
≤ 177.30	**0.571 (0.416, 0.783)**	
> 177.30	1 (-)	
Log(PRC)	**1.107 (1.051, 1.166)**	**<0.001**

Aldosterone

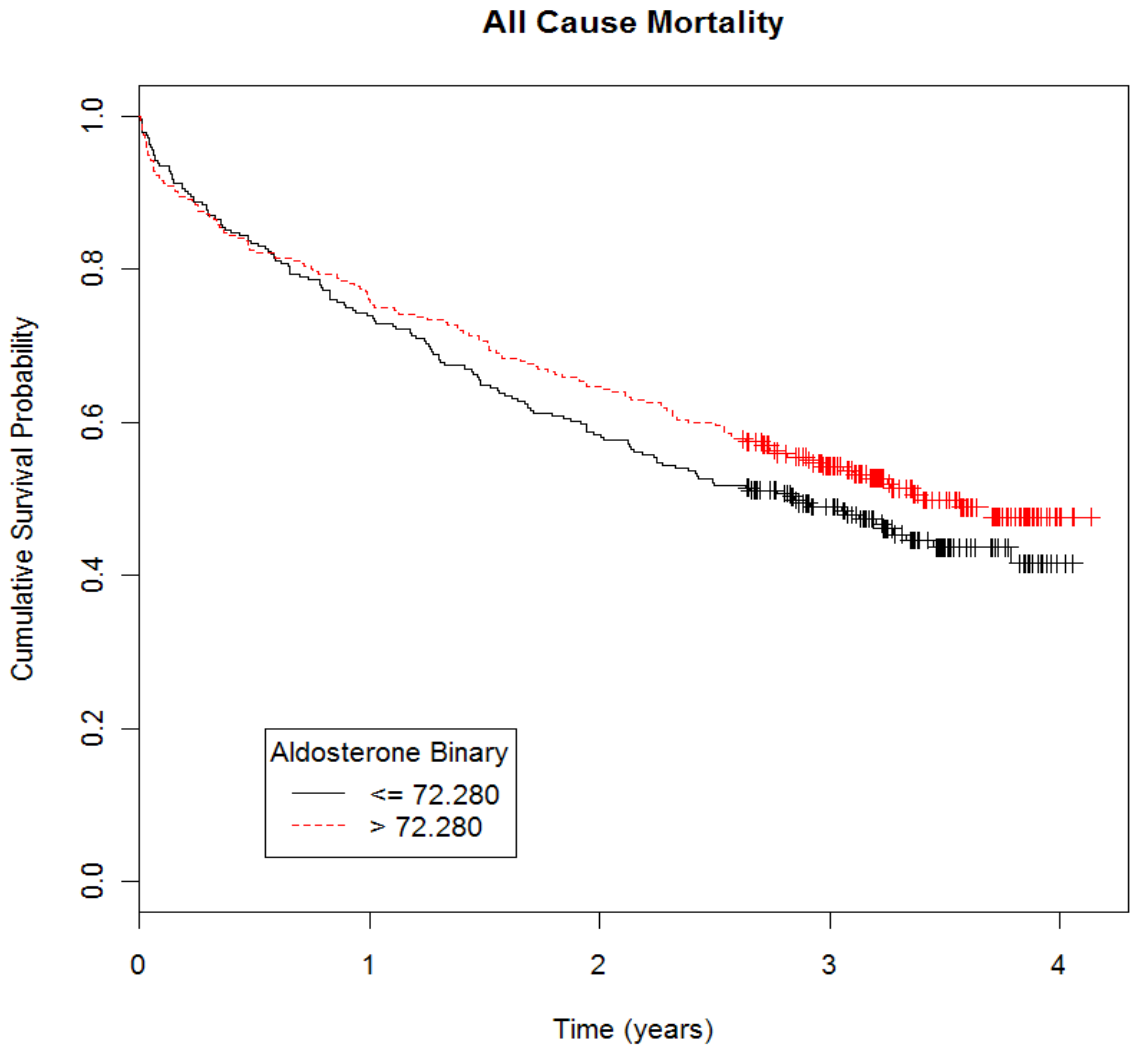
Kaplan-Meier curves for all-cause mortality using aldosterone in quartiles is presented in Figure 10-4. No association of aldosterone with all-cause mortality was seen in this cohort (log-rank p-value = 0.46). There was a trend for worse prognosis in patients with lower aldosterone levels compared to patients with higher aldosterone levels.



	No. at risk				
	Time = 0	1 year	2 year	3 year	4 year
Aldosterone ≤ 31.692	139	99	80	51	2
Aldosterone 31.693 – 72.280	137	105	81	50	1
Aldosterone 72.281 – 151.649	137	108	95	58	3
Aldosterone > 151.649	138	101	83	60	2

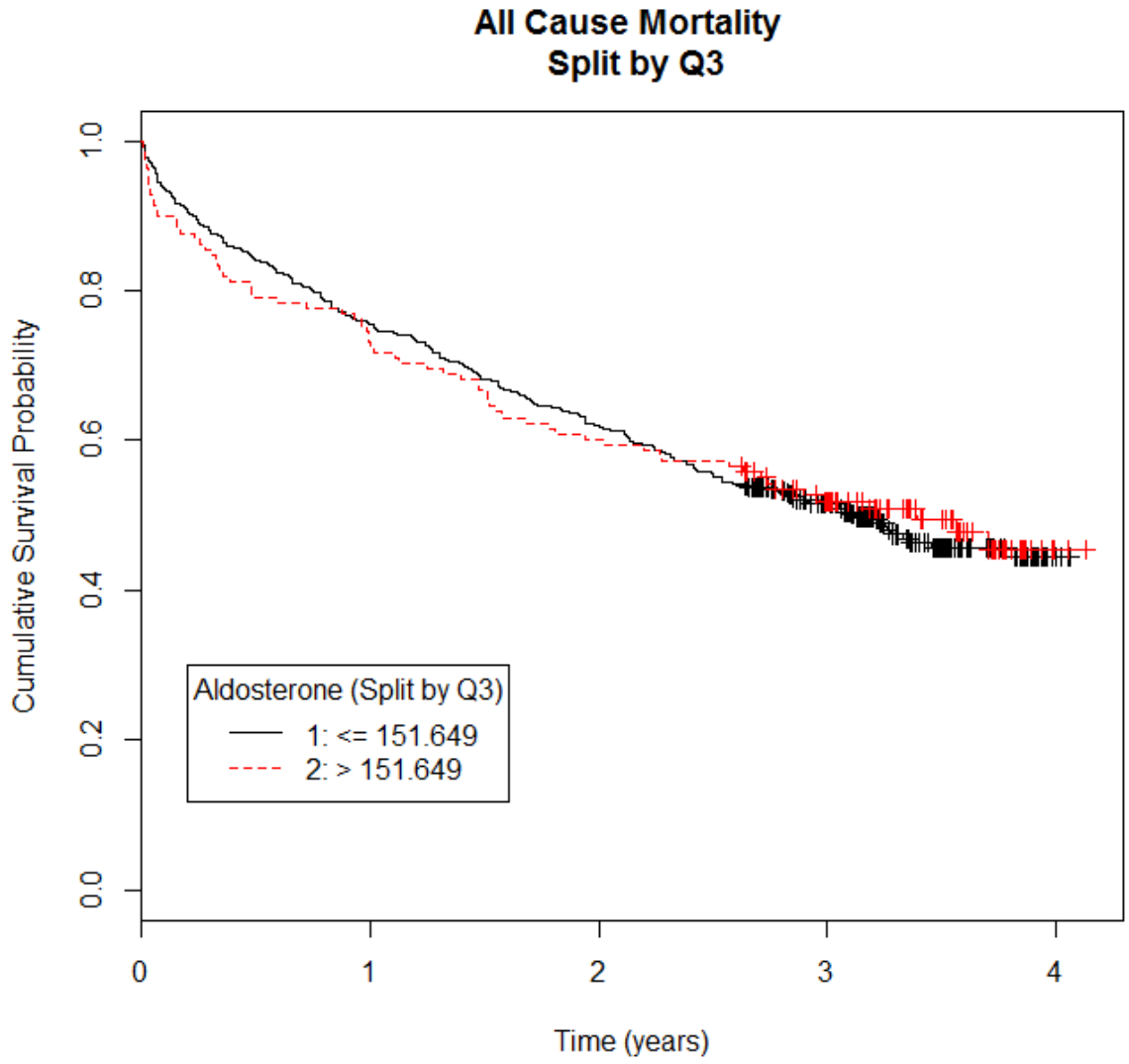
Figure 10-4. Kaplan–Meier event-free curves for patients with decompensated HF according to aldosterone quartiles

Correspondingly, no association of aldosterone with mortality was present when aldosterone was analysed as binary variable according to median (log-rank p-value = 0.185) (Figure 10-5 below) and 75th percentile (log-rank p-value = 0.946) (Figure 10-6).



	No. at risk				
	Time = 0	1 year	2 year	3 year	4 year
Aldosterone ≤ 72.280	276	204	161	101	3
Aldosterone > 72.280	275	209	178	118	5

Figure 10-5. Kaplan–Meier event-free curves for patients with decompensated HF according to median aldosterone



	No. at risk				
	Time = 0	1 year	2 year	3 year	4 year
Aldosterone ≤ 151.649	413	204	161	101	3
Aldosterone > 151.650	138	209	178	118	5

Figure 10-6. Kaplan–Meier event-free curves for patients with decompensated HF according to aldosterone 75th centile

The results of univariate Cox analyses for aldosterone as a predictor of all cause-mortality are displayed in Table 10-3.

Table 10-3. Univariate Cox regression analysis of aldosterone at baseline for all-cause mortality with aldosterone entered as categorised (quartiles/dichotomised according to Q2 and Q3) and continuous (log-transformed variable).

Variable	HR (95% CI)	p-value
Aldosterone (Quartiles) (pmol/L)		0.4626
≤ 31.692	1.123 (0.811, 1.554)	0.4856
31.693 - 72.280	1.055 (0.760, 1.465)	0.7476
72.281 - 151.649	0.863 (0.615, 1.212)	0.3952
> 151.649	1 (-)	
Aldosterone (Split by Q2) (pmol/L)		0.1852
≤ 72.280	1.171 (0.927, 1.479)	
> 72.281	1 (-)	
Aldosterone (Split by Q3) (pmol/L)		0.9465
≤ 151.649	1.009 (0.770, 1.322)	
> 151.650	1 (-)	
Log(Aldosterone)	0.956 (0.895, 1.021)	0.1794

Univariate results for aldosterone as a predictor of all-cause mortality censoring the outcomes at 1 year after the hospital visit are presented in Table 10-4.

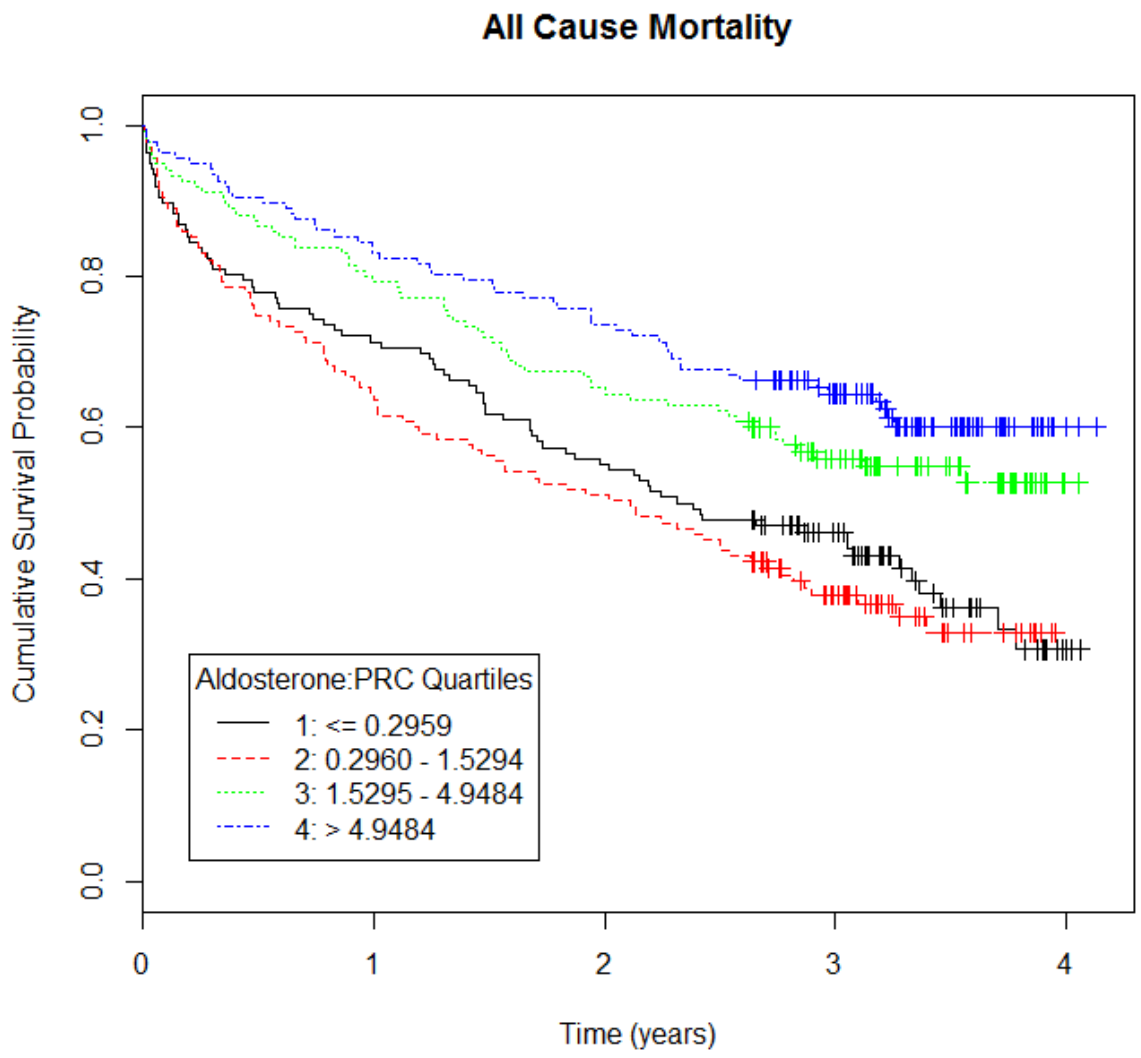
Table 10-4. Univariate Cox regression analysis of aldosterone at baseline for all-cause mortality with aldosterone entered as categorised (quartiles/dichotomised according to Q2 and Q3) and continuous (log-transformed variable) censoring the outcomes at 1 year after hospital admission.

Variable	HR (95% CI)	p-value
Aldosterone (Quartiles) (pmol/L)		0.4782
≤ 31.692	1.060 (0.678, 1.657)	0.7987
31.693 - 72.280	0.840 (0.523, 1.349)	0.4706
72.281 - 151.649	0.753 (0.463, 1.225)	0.2529
> 151.649	1 (-)	
Aldosterone (Split by Q2) (pmol/L)		0.6274
≤ 72.280	1.086 (0.778, 1.517)	
> 72.280	1 (-)	
Aldosterone (Split by Q3) (pmol/L)		**0.0461**
≤ 151.649	**0.791 (0.638, 0.996)**	
> 151.650	1 (-)	
Log(Aldosterone)	0.970 (0.883, 1.066)	0.5273

Aldosterone to PRC ratio

Kaplan-Meier curves for all-cause mortality using the aldosterone to PRC ratio in quartiles are presented in Figure 10-7 (log-rank p-value <0.001). The aldosterone to renin ratio was inversely related with all-cause mortality; patients with the higher aldosterone to PRC ratio had better prognosis than the other groups. However, mortality risk did not decrease in an absolute stepwise inverse fashion across the quartiles of the aldosterone to renin ratio; patients with aldosterone to PRC ratio in the lowest quartile had better prognosis than patients with aldosterone to PRC ratio in the second quartile, who had the worst prognosis.

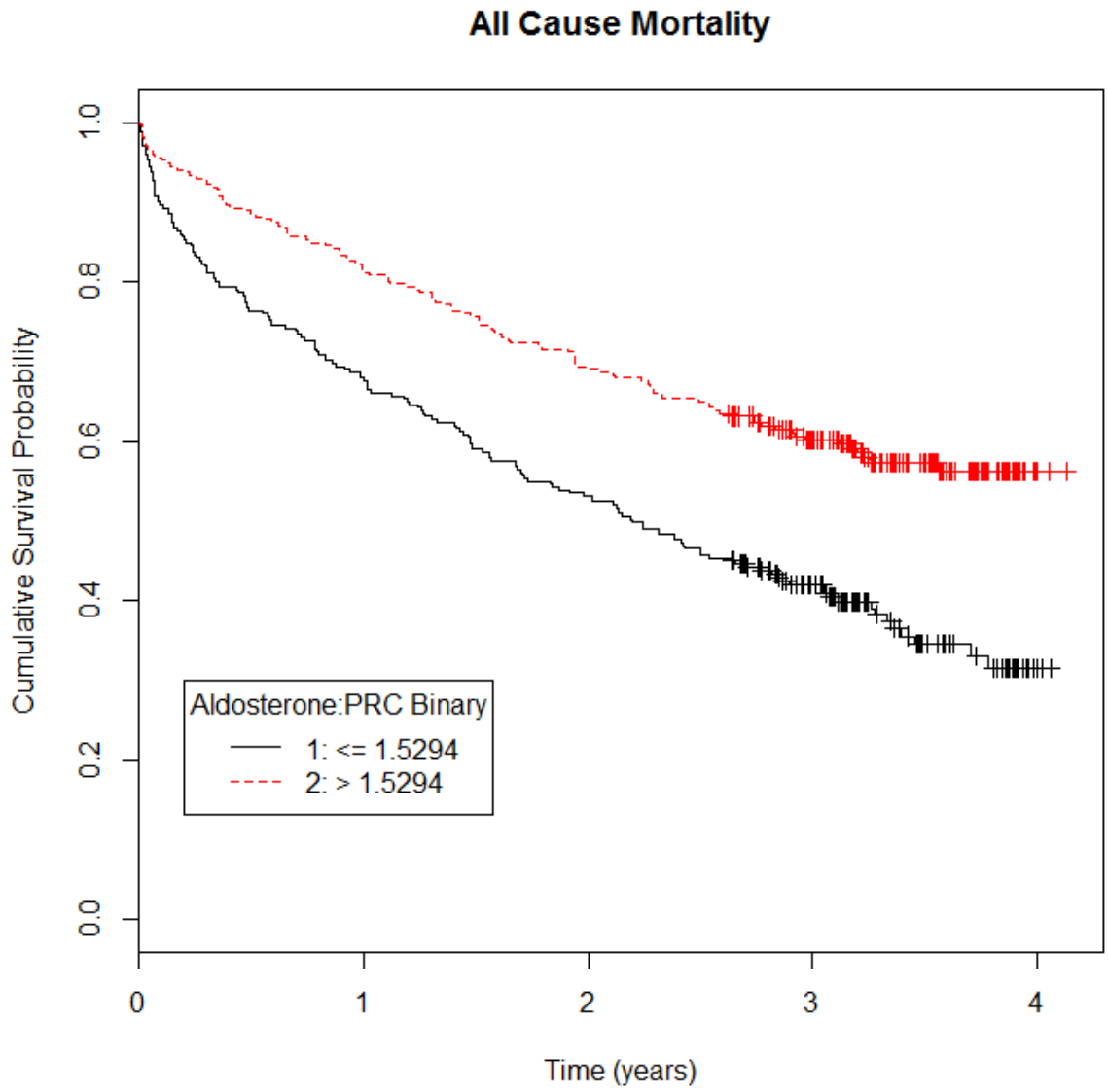
.



	No. at risk				
	Time = 0	1 year	2 year	3 year	4 year
Aldosterone:PRC ≤ 0.2959	136	97	75	45	3
Aldosterone:PRC 0.2960 – 1.5294	135	87	69	38	0
Aldosterone:PRC 1.5295 – 4.9484	135	107	88	58	1
Aldosterone:PRC > 4.9484	136	113	100	72	4

Figure 10-7. Kaplan–Meier event-free curves for patients with decompensated HF according to aldosterone to PRC quartiles

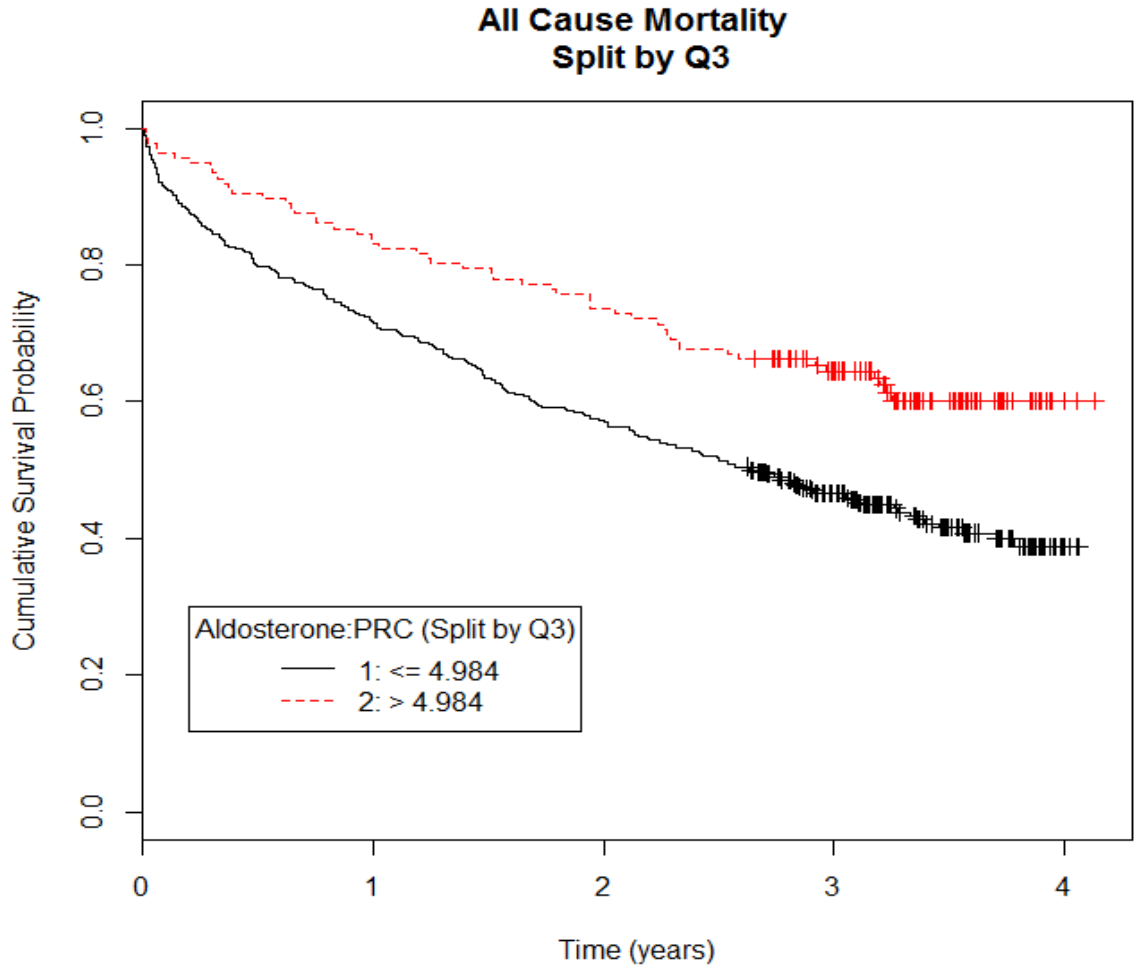
When the aldosterone to PRC ratio was dichotomised according to median, patients with the lowest aldosterone to renin ratio had worse prognosis than patients with the higher aldosterone to PRC ratio (log-rank p-value p <0.001) (Figure 10.8).



	No. at risk				
	Time = 0	1 year	2 year	3 year	4 year
Aldosterone ≤ 1.5294	271	184	144	83	3
Aldosterone > 1.5294	271	220	188	130	5

Figure 10-8. Kaplan–Meier event-free curves for patients with decompensated HF according to median aldosterone to PRC

Likewise, patients with lower aldosterone to PRC ratio had higher mortality rate when the ratio was dichotomised according to the 75th percentile (log-rank p-value <0.001) (Figure 10-9).



	No. at risk				
	Time = 0	1 year	2 year	3 year	4 year
Aldosterone ≤ 4.984	406	291	232	141	4
Aldosterone > 4.984	136	113	100	72	4

Figure 10-9. Kaplan–Meier event-free curves for patients with decompensated HF according to aldosterone to PRC 75th percentile

The univariate analyses of the Cox proportional hazard model for the aldosterone to PRC ratio as a predictor of all-cause mortality as a categorical (quartiles or binary) or continuous variable (log-normalised) are presented in Table 10-5.

Table 10-5. Univariate Cox regression analysis of aldosterone to PRC ratio at baseline for all-cause mortality with aldosterone to PRC ratio entered as categorised (quartiles/ dichotomised according to Q2 and Q3) and continuous (log-transformed variable).

Variable	HR (95% CI)	p-value
Aldosterone:PRC (Quartiles)		**<0.001**
≤ 0.2959	**1.950 (1.377, 2.762)**	**<0.001**
0.2960 – 1.5294	**2.210 (1.565, 3.121)**	**<0.001**
1.5295 – 4.9484	1.273 (0.880, 1.844)	0.2004
4.9484	1 (-)	
Aldosterone:PRC (Split by Q2)	**1.834 (1.444, 2.329)**	**<0.001**
≤ 1.5294	1 (-)	
> 1.5294		
Aldosterone:PRC (Split by Q3)		**<0.001**
≤ 4.984	**1.775 (1.313, 2.399)**	
> 4.984	1 (-)	
Log(Aldosterone:PRC)	**0.877 (0.831 – 0.927)**	**<0.001**

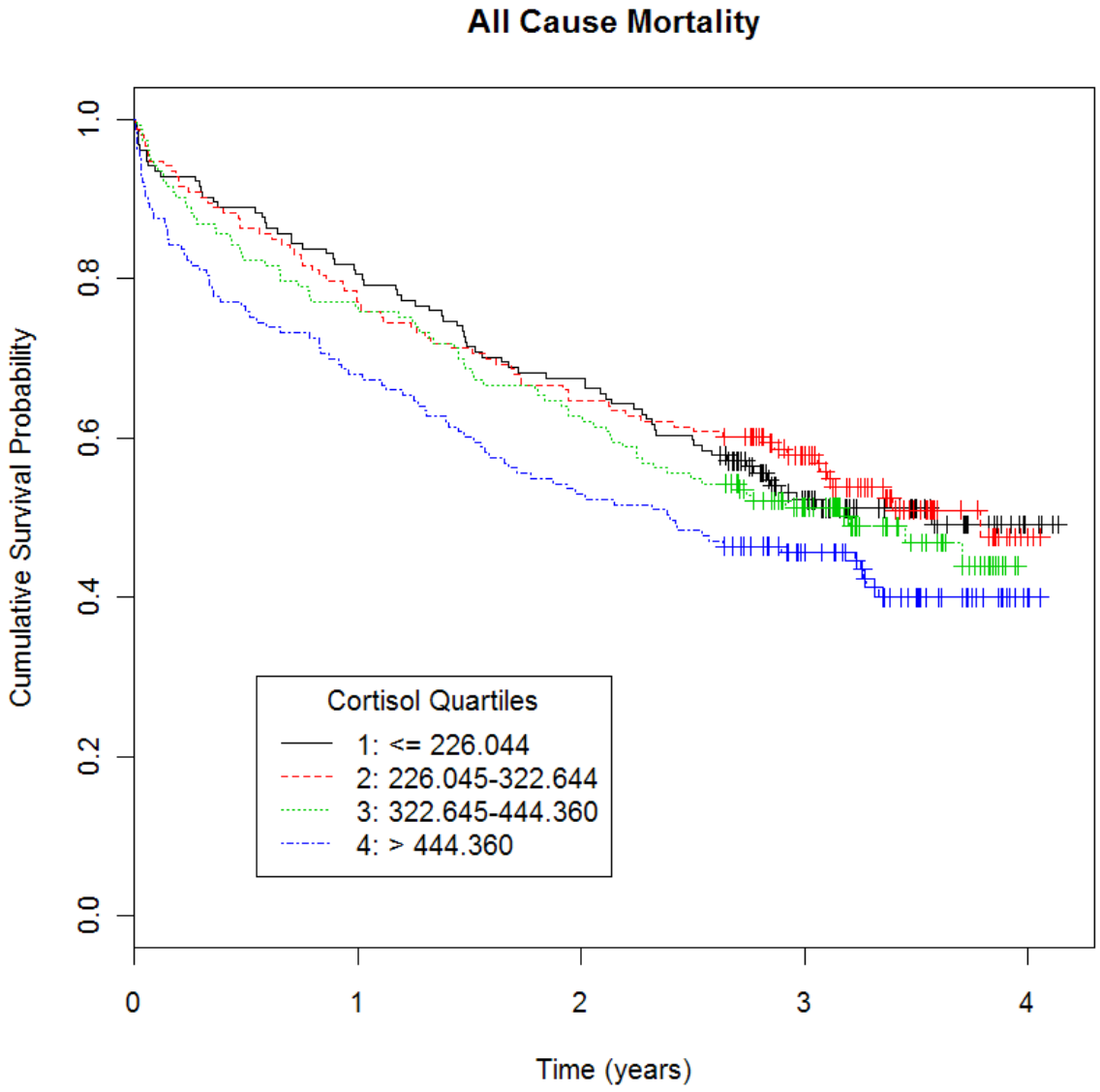
Univariate results for the aldosterone to PRC ratio as a predictor of all-cause mortality at 1 year after the hospital visit are displayed in Table 10-6.

Table 10-6. Univariate Cox regression analysis of aldosterone to PRC ratio at baseline for all-cause mortality with aldosterone to PRC ratio entered as categorised (quartiles/ dichotomised according to Q2 and Q3) and continuous (log-transformed variable) censoring the outcomes at 1 year after hospital admission.

Variable	HR (95% CI)	p-value
Aldosterone:PRC (Quartiles)		**0.0019**
≤ 0.2959	**1.889 (1.128, 3.162)**	**0.0156**
0.2960 – 1.5294	**2.383 (1.450, 3.918)**	**<0.001**
1.5295 – 4.9484	1.261 (0.726, 2.189)	0.4101
4.9484	1 (-)	
Aldosterone:PRC (Split by Q2)		**<0.001**
≤ 1.5294	**1.834 (1.444, 2.329)**	
> 1.5294		
Aldosterone:PRC (Split by Q3)		**0.0085**
≤ 4.984	**1.825 (1.166, 2.865**)	
> 4.984		
Log(Aldosterone:PRC)	**0.923 (0.874, 0.974)**	**0.0038**

Cortisol

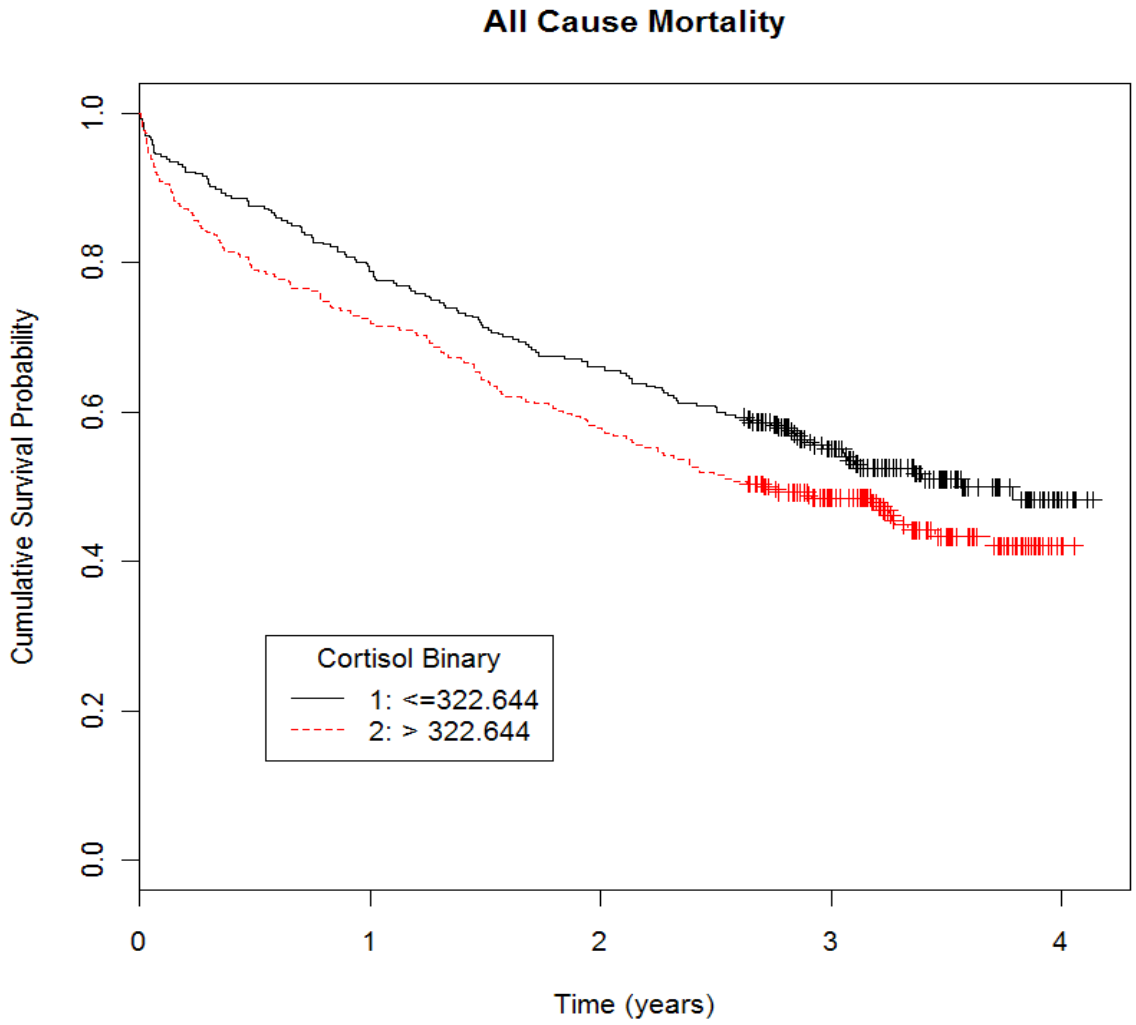
Kaplan-Meier curves for all-cause mortality using cortisol in quartiles is presented in Figure 10-10. Patients with higher cortisol levels had worse prognosis, however that failed to reach statistical significance (log-rank p-value = 0.103).



	No. at risk				
	Time = 0	1 year	2 year	3 year	4 year
Cortisol ≤ 226.044	154	124	104	58	4
Cortisol 226.045 - 322.644	153	118	99	66	4
Cortisol 322.645 - 444.360	153	117	96	57	0
Cortisol > 444.360	153	104	81	52	2

Figure 10-10. Kaplan–Meier event-free curves for patients with decompensated HF according to cortisol quartiles

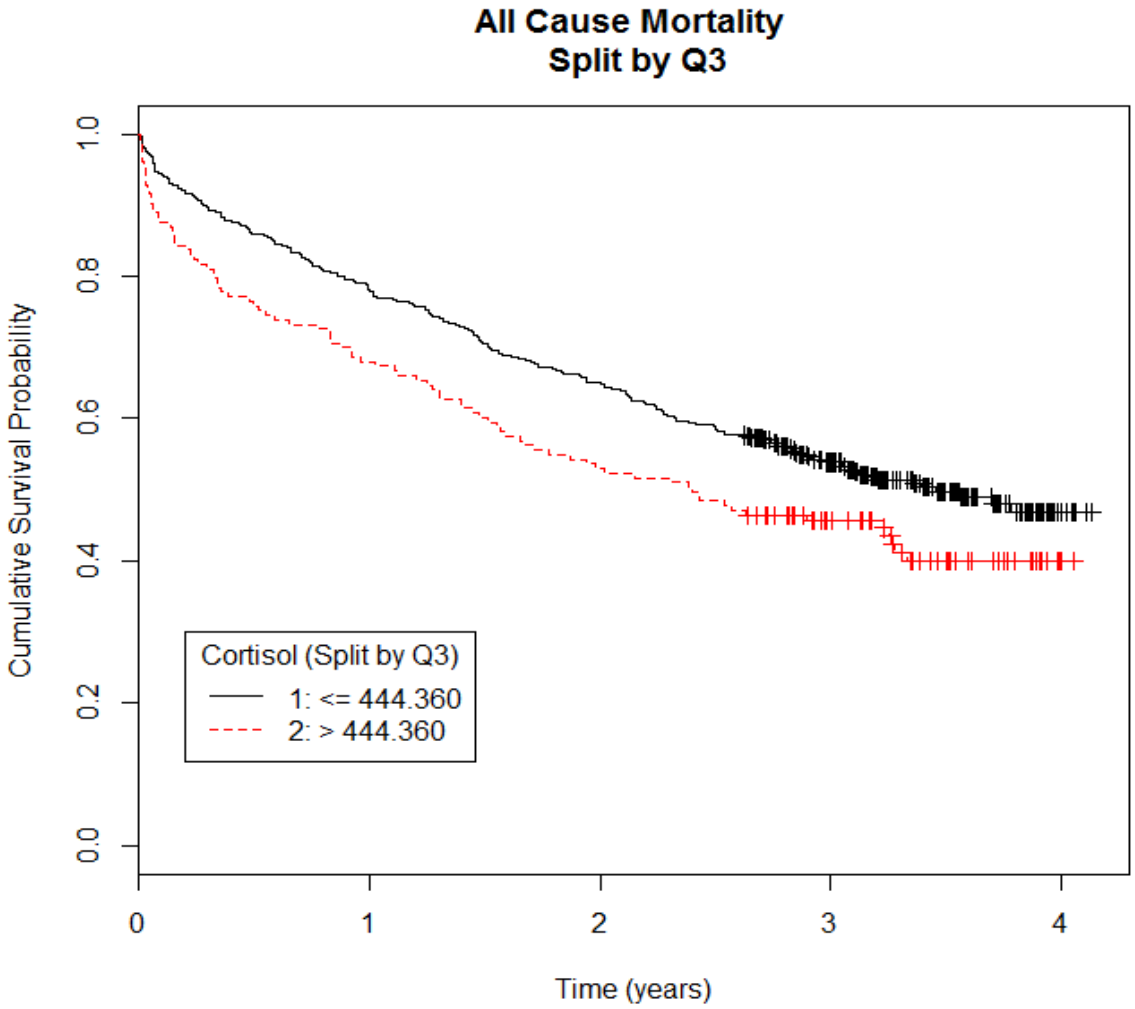
Cortisol was associated with all-cause mortality when analysed as a binary variable according to median; patients with higher cortisol levels were at higher risk of death (log-rank p-value p=0.046). The survival curve for these patients appears to be steeper in the first 6 months after the hospital visit compared to patients with lower cortisol levels; thereafter the hazards appear proportional (Figure 10-11).



	No. at risk				
	Time = 0	1 year	2 year	3 year	4 year
Cortisol ≤ 322.644	307	242	203	124	8
Cortisol > 322.644	306	221	177	109	2

Figure 10-11. Kaplan–Meier event-free curves for patients with decompensated HF according to median cortisol

Similarly, when cortisol was dichotomised according to 75th percentile, patients with higher cortisol levels had worse prognosis (log-rank p-value =0.0193) (Figure 10-12).



	No. at risk				
	Time = 0	1 year	2 year	3 year	4 year
Cortisol ≤ 444.360	460	359	299	181	8
Cortisol > 444.360	153	104	81	52	2

Figure 10-12. Kaplan–Meier event-free curves for patients with decompensated HF according to cortisol 75th percentile

Table 10-7 below summarises the results of Cox univariate analyses of cortisol as a predictor of all-cause mortality analysed as a categorical variable (quartiles or binary) and continuous variable (log transformed).

Table 10-7. Univariate Cox regression analysis of cortisol at baseline for all-cause mortality with cortisol entered as categorised (quartiles/ dichotomised according to Q2 and Q3) and continuous (log-transformed variable).

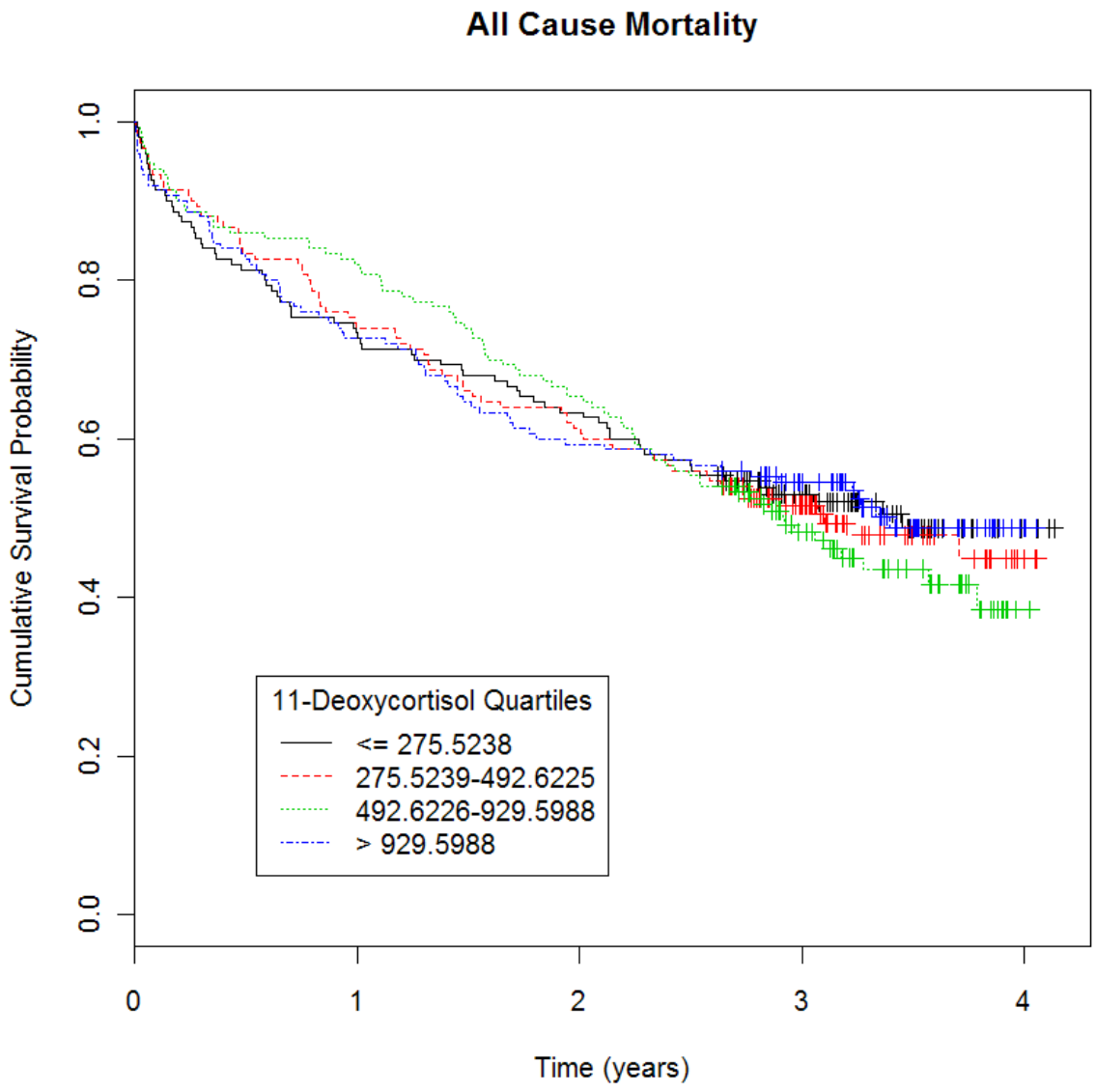
Variable	HR (95% CI)	p-value
Cortisol (Quartiles)(nmol/L)		0.1049
≤ 226.044	**0.730 (0.536, 0.995)**	**0.0463**
226.045 - 322.644	**0.702 (0.514, 0.960)**	**0.0268**
322.645 - 444.360	0.807 (0.595, 1.094)	0.1670
> 444.360	1.00 (-)	
Cortisol (Split by Q2) (nmol/L)		**0.046**
≤ 322.644	**0.797 (0.638, 0.996)**	
> 322.644	1.00 (-)	
Cortisol (split by Q3) (nmol/L)		**0.0197**
≤ 444.360	**0.746 (0.583, 0.954)**	
> 444.360	1.00 (-)	
Log(Cortisol)	1.066 (0.896, 1.268)	0.4738

Univariate results for cortisol as a predictor of all-cause mortality censoring the outcomes at 1 year after hospital admission are displayed in Table 10-8.

Table 10-8. Univariate Cox regression analysis of cortisol at baseline for all-cause mortality with cortisol entered as categorised (quartiles/ dichotomised according to Q2 and Q3) and continuous (log-transformed variable) censoring the outcomes at 1 year after hospital admission.

Variable	HR (95% CI)	p-value
Cortisol (Quartiles) (nmol/L)		**0.0465**
≤ 226.044	**0.548 (0.348, 0.863)**	**0.0095**
226.045 - 322.644	0.648 (0.420, 1.001)	0.0504
322.645 - 444.360	0.682 (0.444, 1.049)	0.0815
> 444.360	1.00 (-)	
Cortisol (Split by Q2) (nmol/L)		**0.043**
≤ 322.644	**0.716 (0.518, 0.989)**	
> 322.644	1.00 (-)	
Cortisol (split by Q3) (nmol/L)		**0.0071**
≤ 444.360	**0.626 (0.445, 0.880)**	
> 444.360	1 (-)	
Log(Cortisol)	1.227 (0.939, 1.603)	0.1347

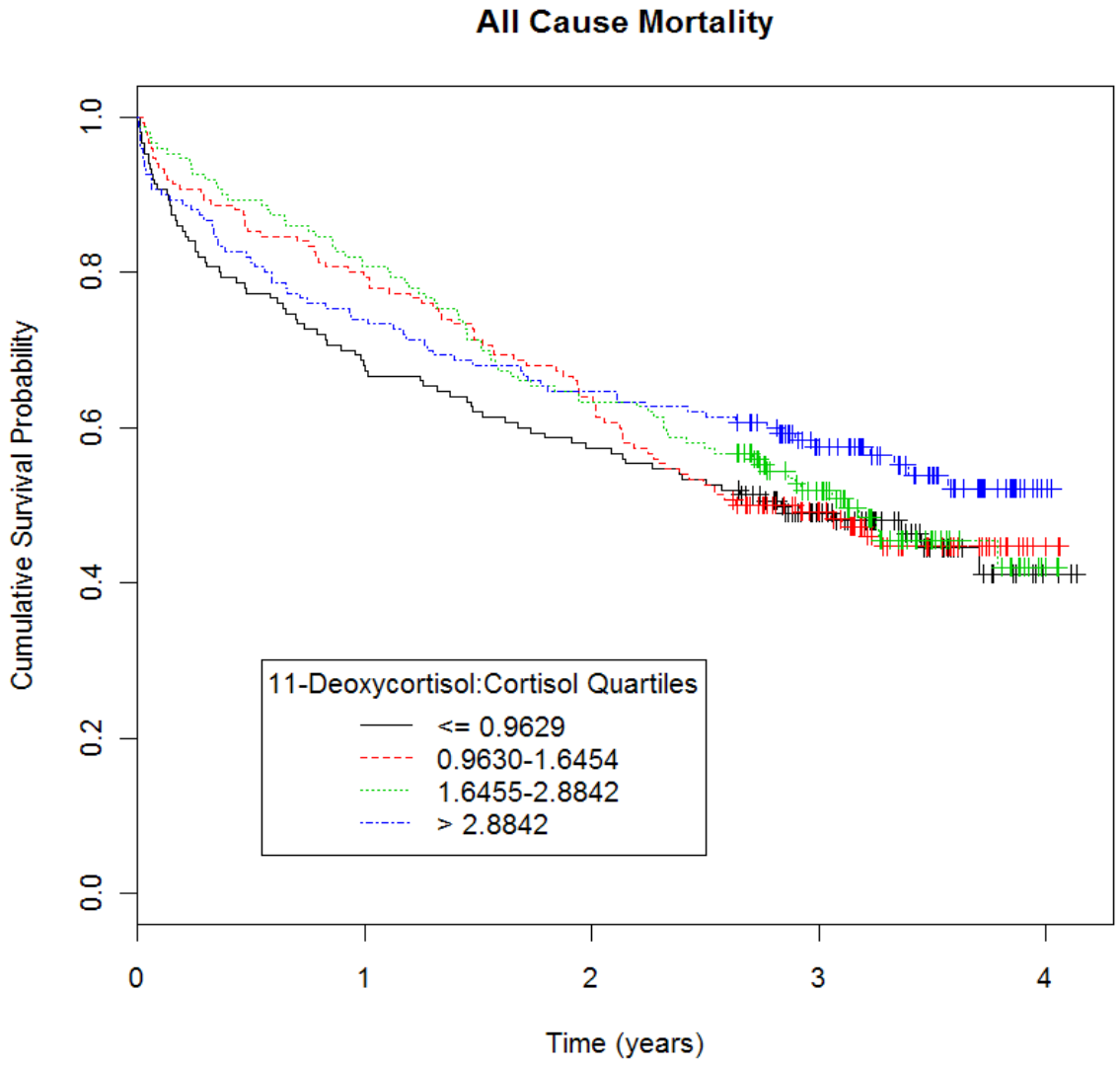
No association was found between 11-deoxycortisol and mortality (log-rank p = 0.941) (Figure 10-13).



	No. at risk				
	Time = 0	1 year	2 year	3 year	4 year
11-Deoxycortisol ≤ 275.5238	150	110	95	58	4
11-Deoxycortisol 275.5239 – 492.6225	150	111	92	55	3
11-Deoxycortisol 492.6226 – 929.5988	150	123	98	51	1
11-Deoxycortisol > 929.5988	150	109	89	64	2

Figure 10-13. Kaplan–Meier event-free curves for patients with decompensated HF according to 11-deoxycortisol quartiles

Similar to 11-deoxycortisol, the 11-deoxycortisol to cortisol ratio was not associated with prognosis (log-rank p-value = 0.427) (Figure 10-14).



	No. at risk				
	Time = 0	1 year	2 year	3 year	4 year
11-Deoxycortisol:Cortisol ≤ 0.9629	150	102	86	53	3
11-Deoxycortisol:Cortisol 0.9630 – 1.6454	150	119	96	55	3
11-Deoxycortisol:Cortisol 1.6455 – 2.8842	150	121	95	57	2
11-Deoxycortisol:Cortisol > 2.8842	150	111	97	63	2

Figure 10-14. Kaplan–Meier event-free curves for patients with decompensated HF according to the 11-deoxycortisol to cortisol ratio quartiles

10.3.3 Multivariate predictors of all-cause mortality

To examine further if cortisol and renin confer prognostic value over and above established prognostic factors in patients with decompensated HF, they were included individually into models, which included independent predictors of all-cause mortality in the overall population identified in multivariable models with predefined variables not including renin or cortisol. These independent predictors were age, SBP, previous history of COPD, haemoglobin, urea, troponin, log(BNP) for the models had PRC added in and age, SBP, previous history of COPD, previous hospitalisation with HF, haemoglobin, urea, troponin and log(BNP) for the models that included cortisol.

PRC

When PRC was included in multivariate Cox analysis as a quartile, there was an overall trend for higher of all-cause mortality in patients with higher PRC levels (p=0.0545) with a HR of 0.6433 (95% CI , 0.4457 to 0.9284) for lowest versus highest PRC quartile and a HR of 0.6591 (95% CI , 0.4688, 0.9627) for patients in the second PRC quartile compared with patients in the highest PRC quartile (Table 10-9)**.** Further analysis with PRC analysed as binary variable according to median and 75th percentile showed a positive association of PRC with all-cause mortality. Similarly, PRC as a continuous variable (log-transformed) was associated with death; an increase of 1 unit of log(PRC) was correlated with approximately 8% higher risk of all-cause mortality. Other independent predictors of all-cause mortality in these models were increasing age, lower SBP and haemoglobin, history of COPD and elevated urea and BNP (Table 13-13 to Table 13-16 in the Appendix).

Table 10-9. Univariate and Multivariate Cox proportional hazard ratios (95% CI) for all-cause mortality according to PRC entered as quartiles, binary (split by Q2 and Q3) and continuous (log-transformed) variable.

Variable	Univariate HR (95% CI), P-value	Multivariate HR (95% CI), P-value
PRC (Quartiles) (mIU/L)	**<0.0001**	0.0545
≤13.00	**0.4743 (0.3506, 0.66416), <0.0001**	**0.6433 (0.4457, 0.9284), 0.0184**
13.01 – 47.30	**0.5884 (0.4408, 0.7852), 0.0003**	**0.6591 (0.4688, 0.9627), 0.0165**
47.31 – 177.30	**0.7296 (0.5543, 0.9604), 0.0246**	0.8143 (0.5932, 1.1177), 0.2036
>177.30	1 (-)	1 (-)
PRC ≤47.30 (mIU/L)	**0.6196 (0.5012, 0.7569), <0.0001**	**0.7241 (0.5586, 0.9387), 0.0148**
PRC ≤177.30 (mIU/L)	**0.5931 (0.4735, 0.7427), <0.0001**	**0.7178 (0.5480, 0.9402), 0.0161**
Log(PRC)	**1.1050 (1.0649, 1.1467), <0.0001**	**1.0766 (1.0259, 1.1298), 0.0027**

Unadjusted and adjusted Kaplan-Meier plots for all-cause mortality using PRC in quartiles and as a binary variable (split by Q2 and Q3) are displayed in Figure 10-15 to Figure 10-17.

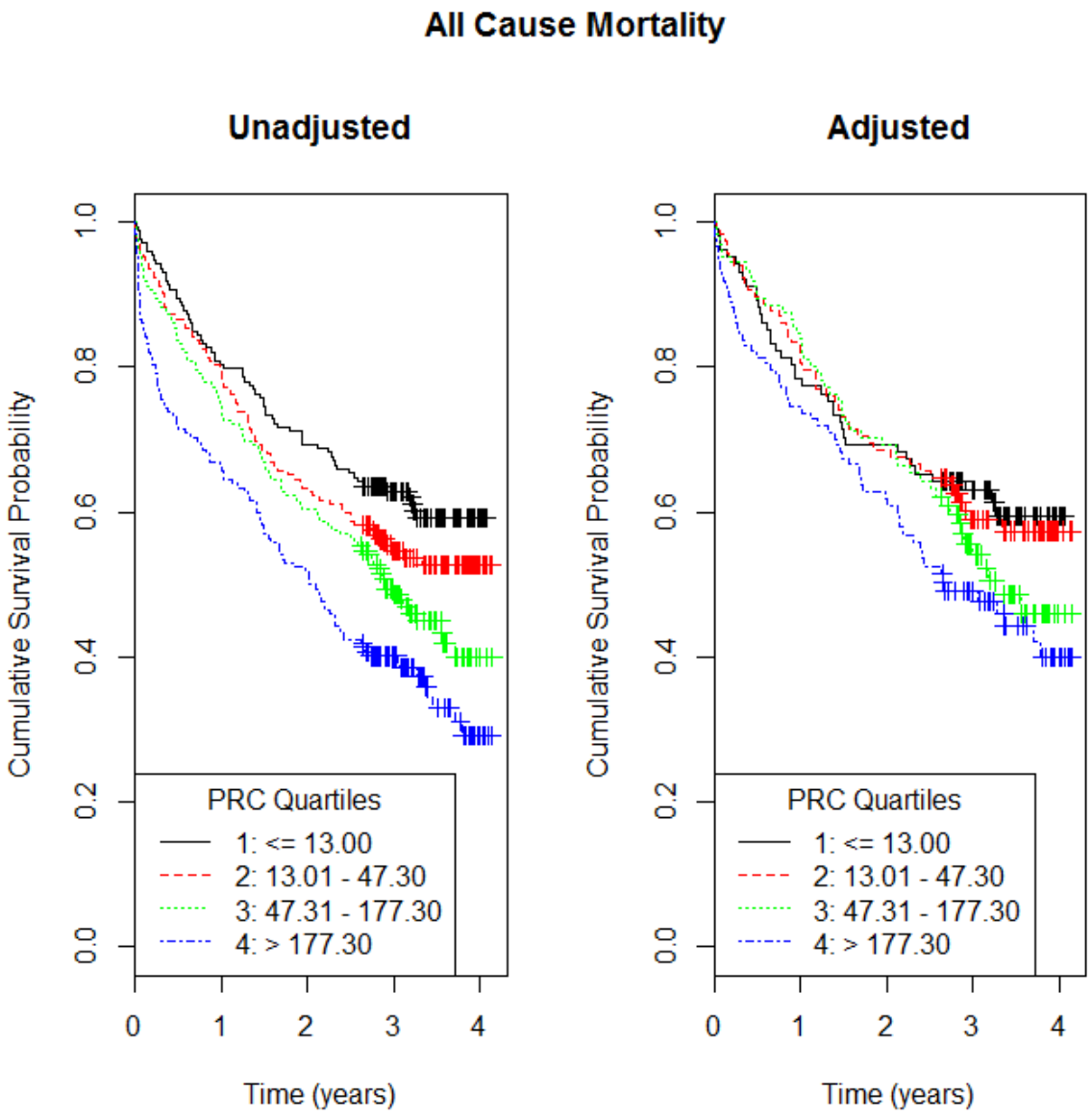


Figure 10-15. Unadjusted and adjusted Kaplan-Meier curves for all-cause mortality according to PRC (quartiles) at baseline

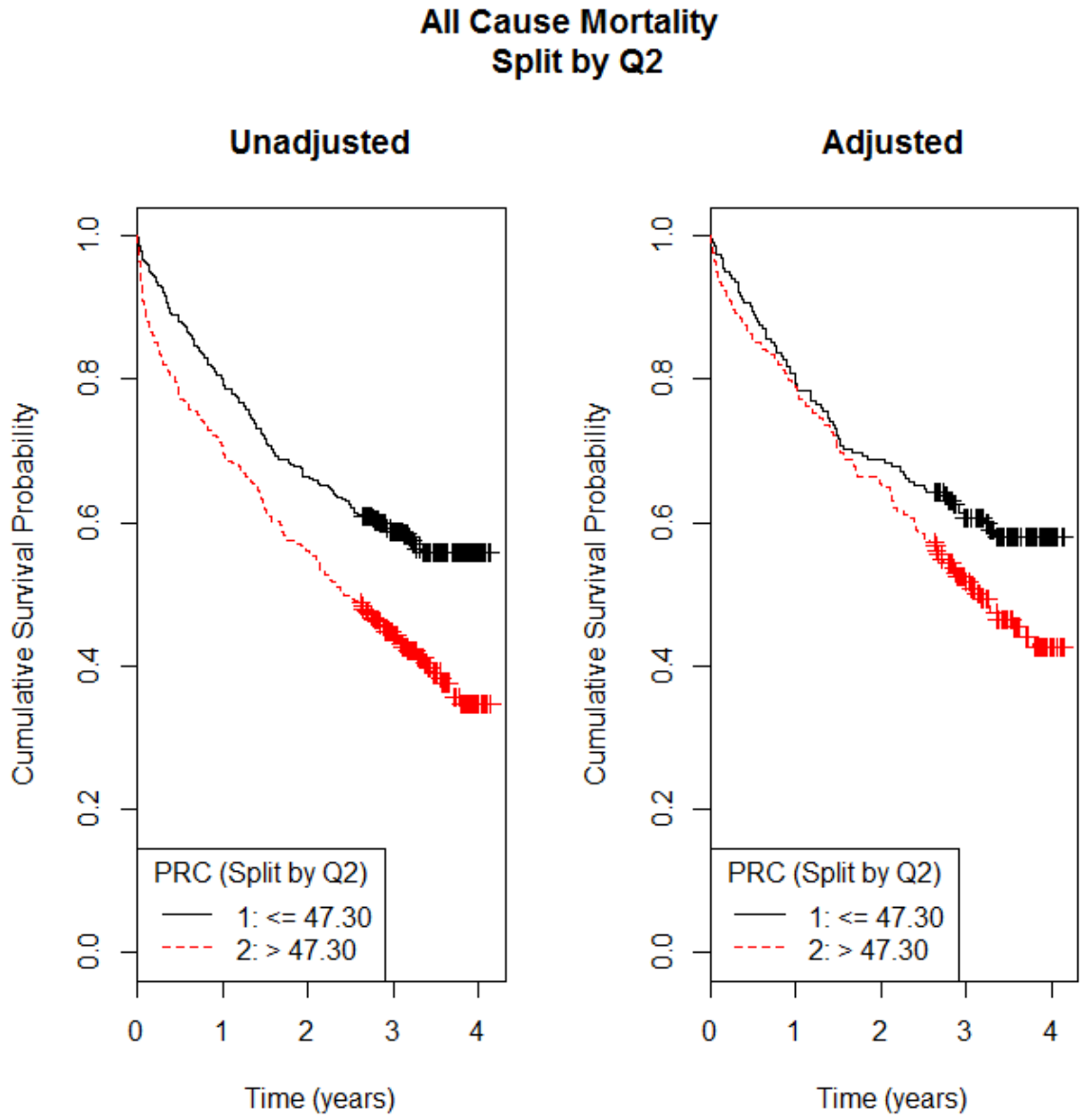


Figure 10-16. Unadjusted and adjusted Kaplan-Meier curves for all-cause mortality according to PRC (dichotomised according to median,Q2) at baseline

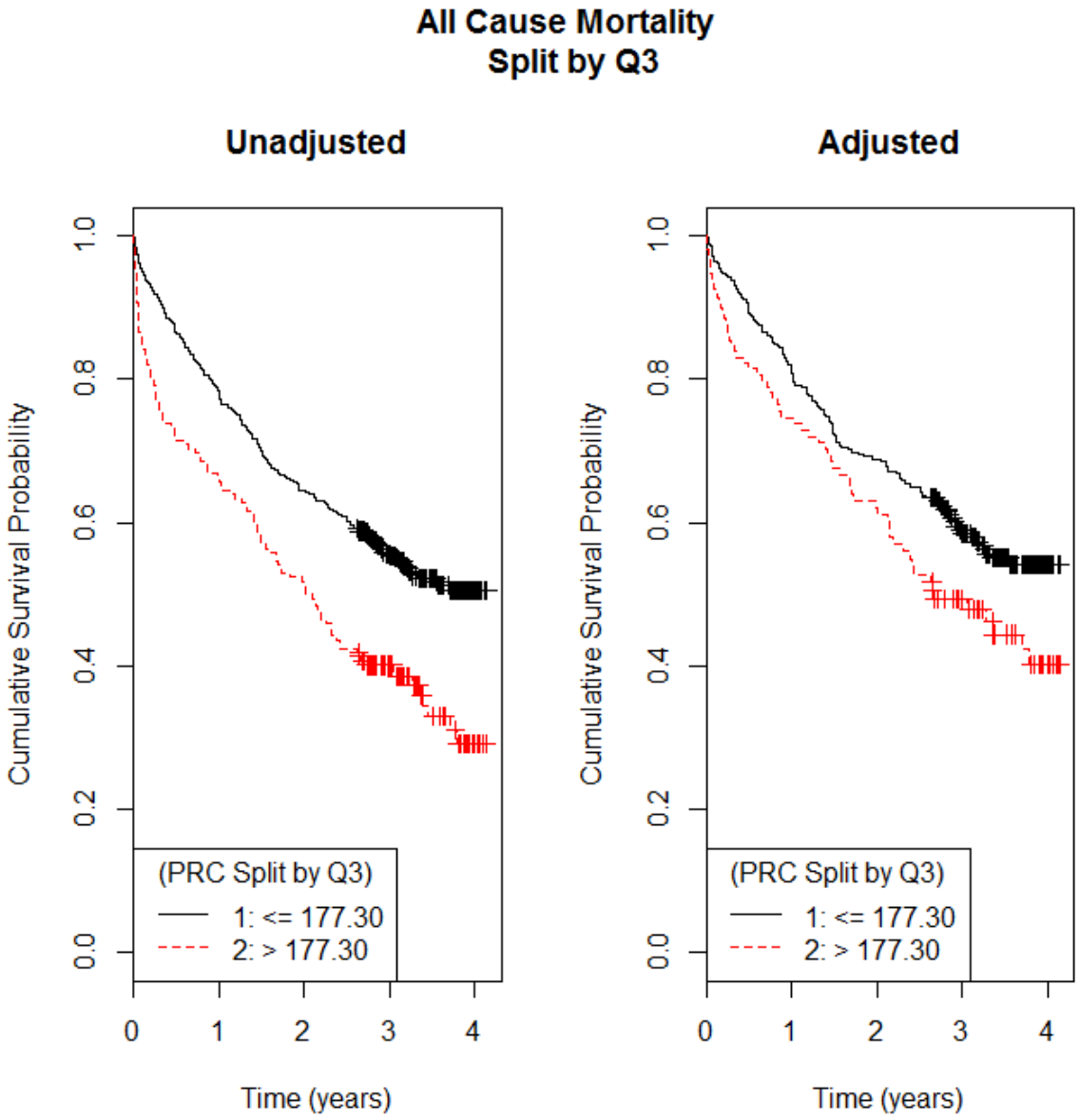


Figure 10-17. Unadjusted and adjusted Kaplan-Meier curves for all-cause mortality according to PRC (dichotomised according to 75th centile, Q3) at baseline

Censoring at 1 year after hospital admission

In contrast to the prognostic value of PRC for the full follow-up, no prognostic significance was identified when PRC was included in a Cox multivariate model censoring at 1 year after hospital admission (Table 10-10).

Table 10-10. Univariate and Multivariate Cox proportional hazard ratios (95% CI) for all-cause mortality at 1 year after hospital admission according to PRC entered as quartile, binary (split by Q2 and Q3) and continuous (log-transformed) variable.

Variable	Univariate HR (95% CI), P-value	Multivariate HR (95% CI), P-value
PRC (Quartiles) (mIU/L)	**0.0038**	0.4749
≤13.00	**0.4974 (0.3257, 0.7597), 0.0012**	0.7160 (0.4480, 1.1444), 0.1627
13.01 – 47.30	**0.5562 (0.3682, 0.8402), 0.0053**	0.7564 (0.4902, 1.1674), 0.2073
47.31 – 177.30	**0.6629 (0.4468, 0.9835), 0.0411**	0.7990 (0.5320, 1.1999), 0.2794
>177.30	1 (-)	1 (-)
PRC ≤47.30 (mIU/L)	**0.6405 (0.4728, 0.8677), 0.0040**	0.8276 (0.5961, 1.1492), 0.2586
PRC ≤177.30 (mIU/L)	**0.5708 (0.4160, 0.7831), 0.0005**	0.7645 (0.5422, 1.0781), 0.1257
Log(PRC)	**1.1080 (1.0151, 1.1677), 0.0001**	1.0572 (0.9956, 1.1227), 0.0696

Kaplan Meier curves for PRC censoring at 1 year unadjusted and adjusted for independent prognostic markers are displayed in Figure 10-18 to Figure 10-20.

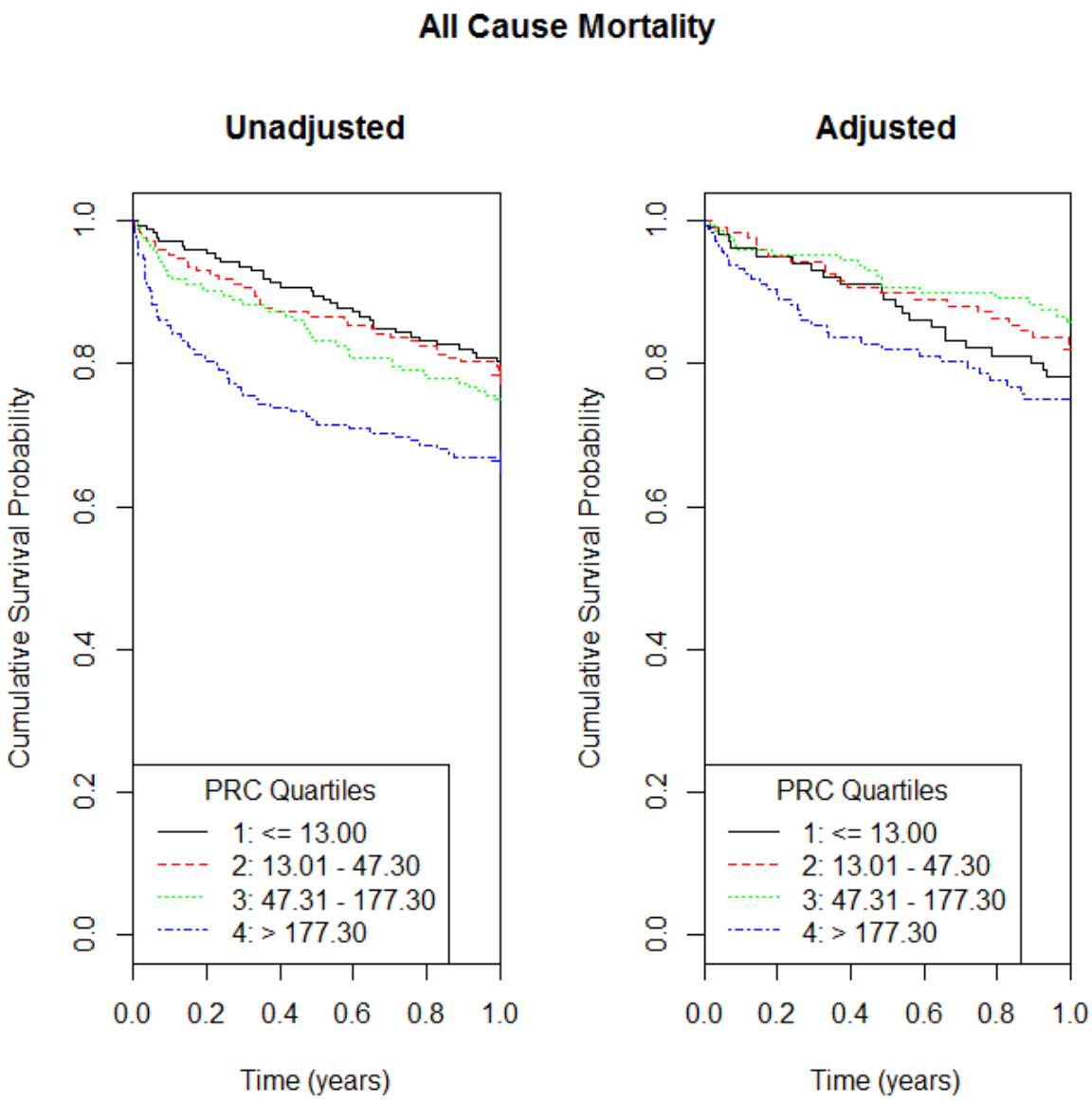


Figure 10-18. Unadjusted and adjusted Kaplan-Meier curves for all-cause mortality at 1 year after hospital admission according to PRC (quartiles) at baseline

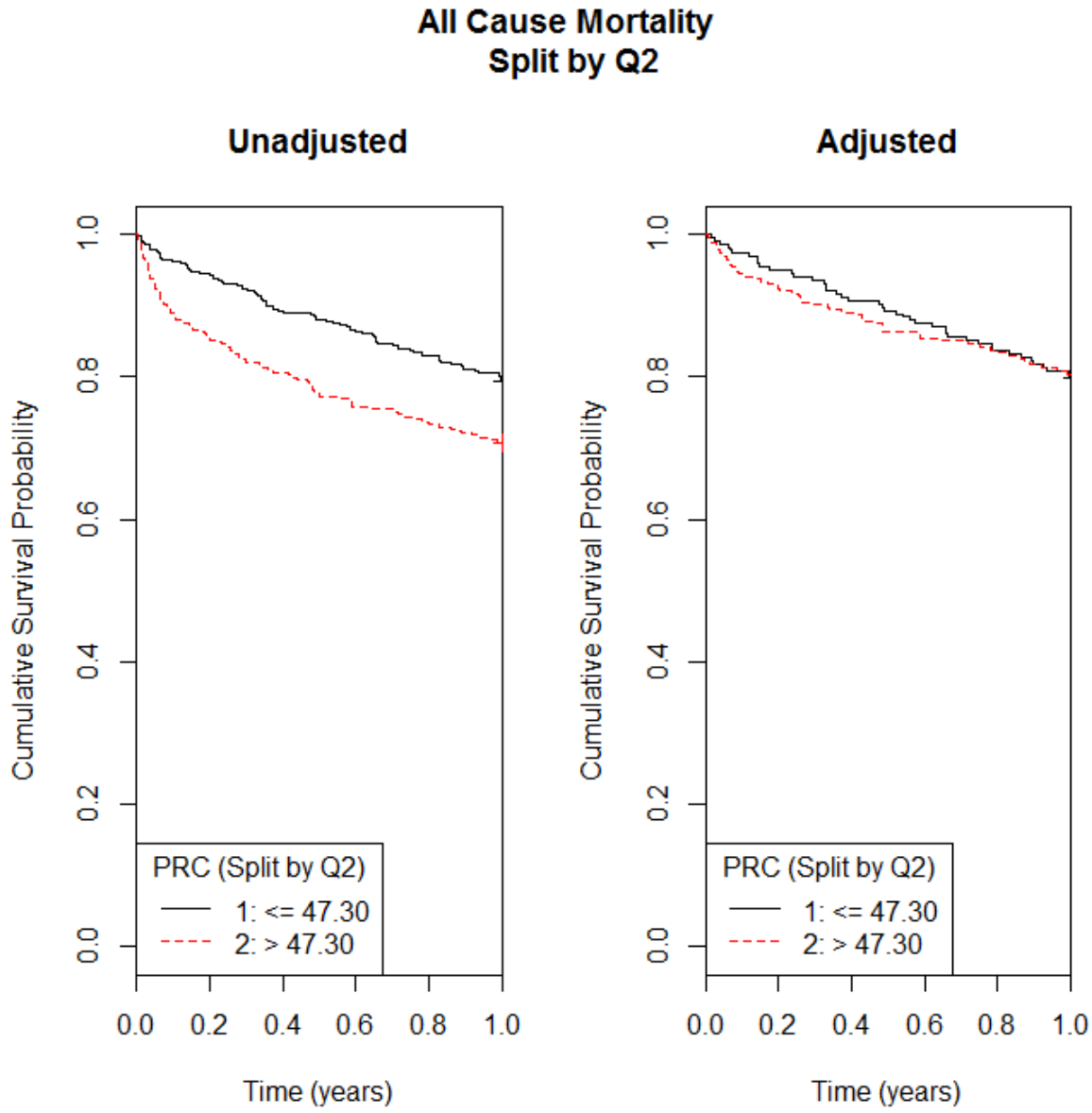


Figure 10-19. Unadjusted and adjusted Kaplan-Meier curves for all-cause mortality at 1 year after hospital admission according to PRC (dichotomised according to median, Q2) at baseline

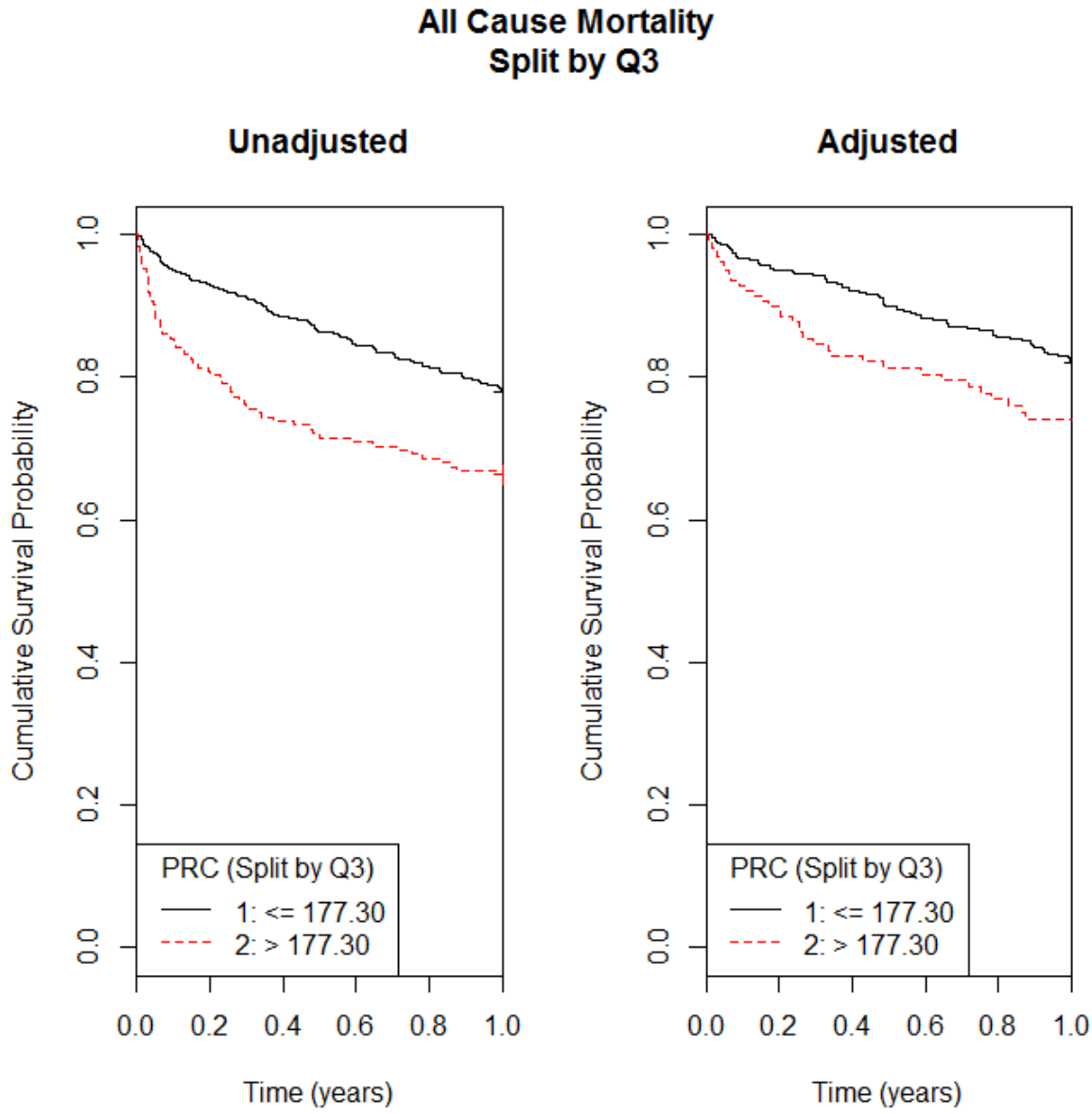


Figure 10-20. Unadjusted and adjusted Kaplan-Meier curves for all-cause mortality at 1 year after hospital admission according to PRC (dichotomised according to 75th centile, Q3) at baseline

Cortisol

When Cortisol was considered along with independent prognostic factors in a Cox multivariate regression analysis, expressed either as quartiles, binary (split by median and 75th centile) variable or continuous (log-transformed) variable, it was not associated with all-cause mortality (Table 10-11). The independent predictors of all-cause mortality in these models were age, SBP, history of previous HF hospitalisation, history of COPD, urea, troponin and log(BNP) (Table 13-17 to Table 13-20 in the Appendix).

Table 10-11. Univariate and Multivariate Cox proportional hazard ratios (95% CI) for all-cause mortality according to cortisol entered as quartiles, binary (split by Q2 and Q3) and continuous (log-transformed) variable.

Variable	Univariate HR (95% CI), P-value	Multivariate HR (95% CI), P-value
Cortisol (Quartiles) (nmol/L)	0.1051	0.1221
≤226.644	**0.7303 (0.5361, 0.9950), 0.0464**	1.0299 (0.7283, 1.4534), 0.8677
226.045 – 322.644	**0.7025 (0.5138, 0.9604), 0.0269**	**0.6798 (0.9408, 0.9920), 0.0450**
322.645 – 444.360	0.8066 (0.5974, 1.0942), 0.1671	0.8929 (0.6284, 1.2687), 0.5273
>444.360	1 (-)	1 (-)
Cortisol ≤322.644 (nmol/L)	**0.7971 (0.6379, 0.9961), 0.0461**	0.8825 (0.6843, 1.1381), 0.3354
Cortisol ≤444.360 (nmol/L)	**0.7456 (0.5825, 0.9543), 0.0197**	0.8554 (0.6474, 1.1302), 0.2718
Log(Cortisol)	1.0657 (0.8955, 1.2682), 0.4738	0.9251 (0.7643, 1.1198), 0.4245

Kaplan-Meier plots for all-cause mortality using cortisol in quartiles and as a binary variable (both split by Q2 and Q3) are presented in Figure 10-21 to Figure 10-23.

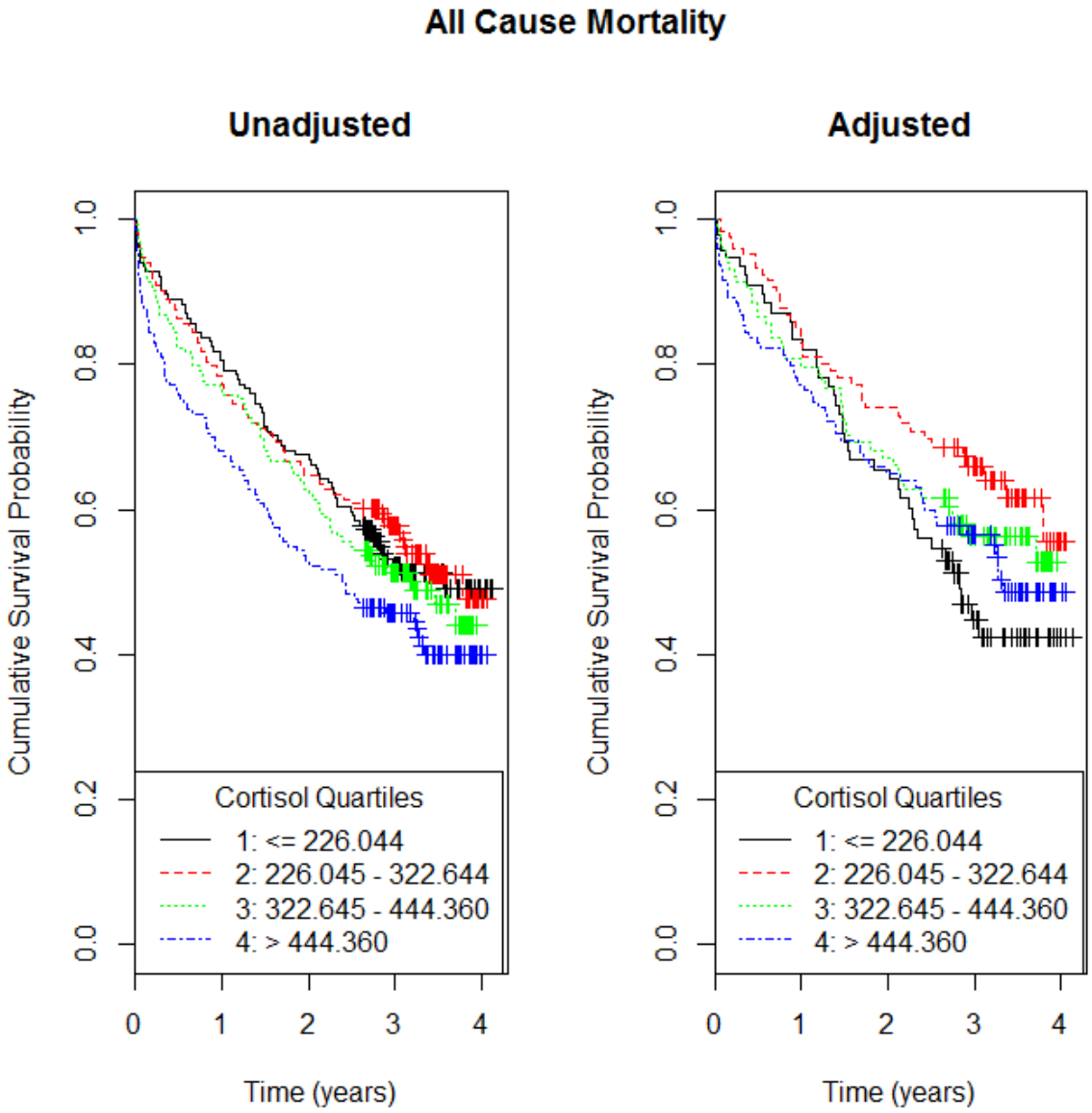


Figure 10-21. Unadjusted and adjusted Kaplan-Meier curves for all-cause mortality according to quartiles of plasma cortisol at baseline

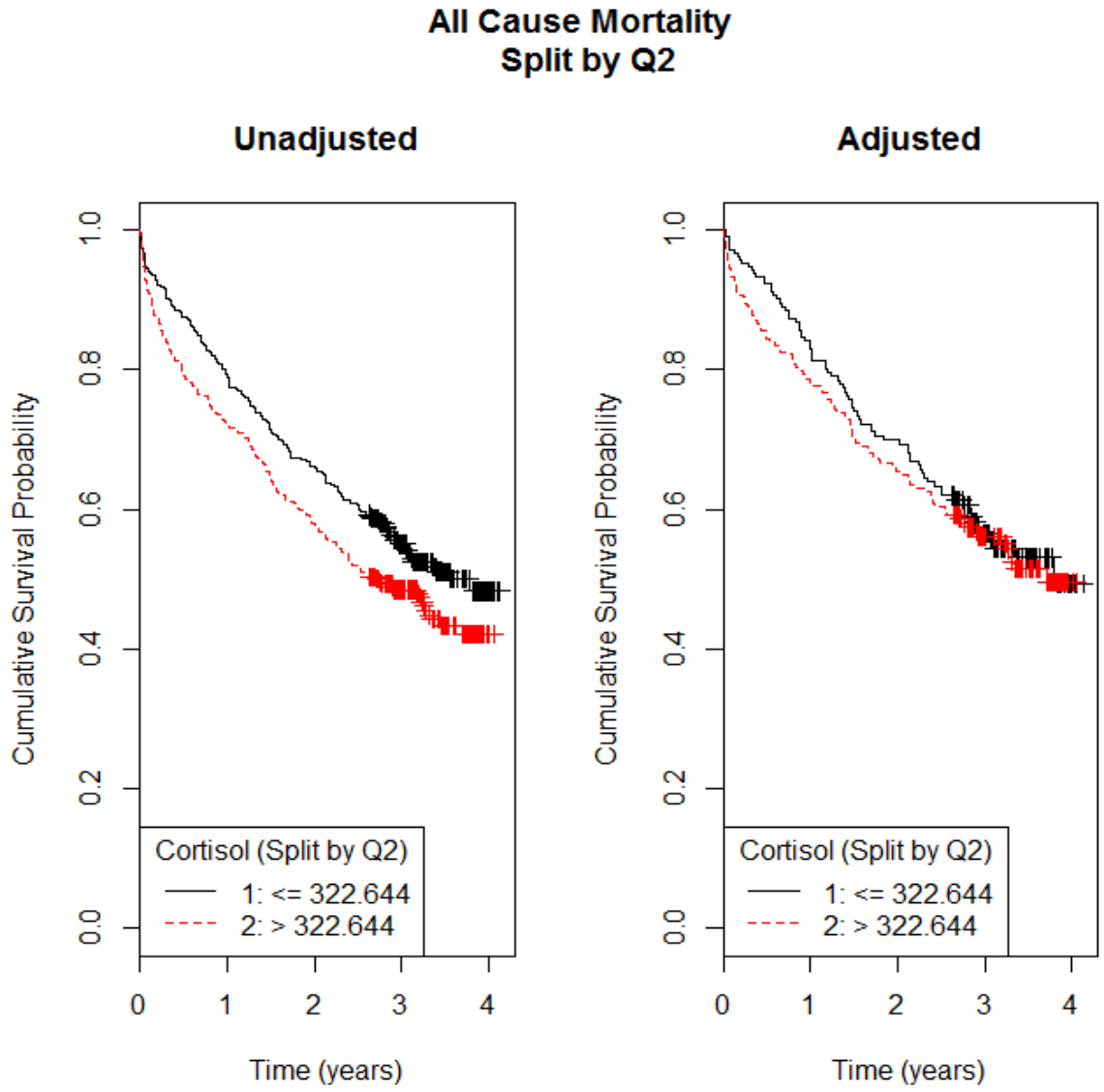


Figure 10-22. Unadjusted and adjusted Kaplan-Meier curves for all-cause mortality according to plasma cortisol (dichotomised according to median, Q2) at baseline

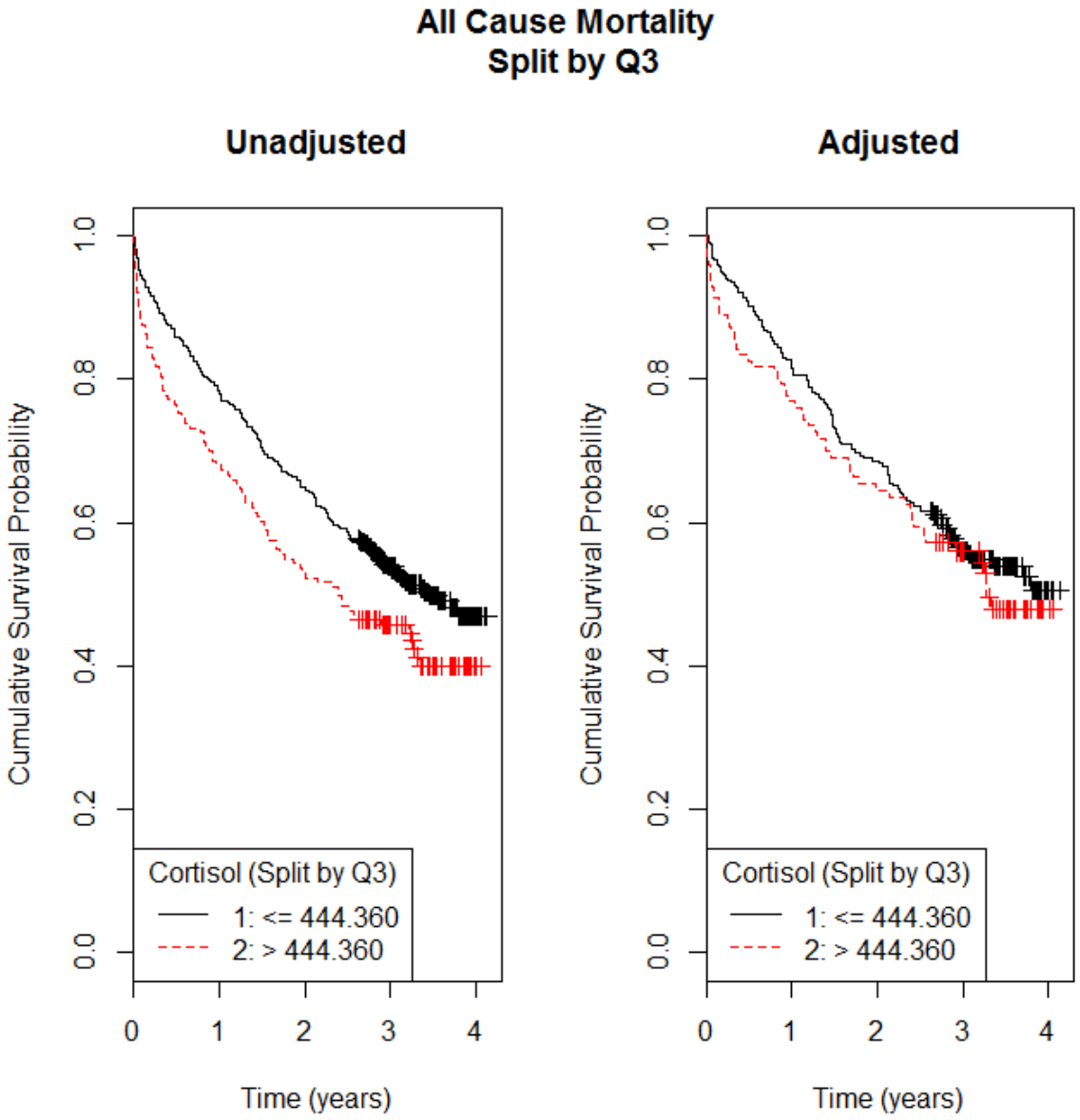


Figure 10-23. Unadjusted and adjusted Kaplan-Meier curves for all-cause mortality according to plasma cortisol (dichotomised according to 75th centile, Q3) at baseline

Censoring at 1 year after hospital admission

Similarly, when cortisol was included in a Cox multivariate model censoring at 1 year after hospital admission no prognostic importance was elucidated for this glucocorticoid (Table 10-12).

Table 10-12. Univariate and Multivariate Cox proportional hazard ratios (95% CI) for all-cause mortality at 1 year after hospital admission according to cortisol entered as quartiles, binary (split by Q2 and Q3) and continuous (log-transformed) variable.

Variable	Univariate HR (95% CI), P-value	Multivariate HR (95% CI), P-value
Cortisol (Quartiles) (nmol/L)	**0.0468**	0.5139
≤226.644	**0.5482 (0.3480, 0.8635), 0.0095**	0.7296 (0.4380, 1.2153), 0.2260
226.045 – 322.644	0.6487 (0.4204, 1.0010), 0.0505	0.7472 (0.4590, 1.2162), 0.2410
322.645 – 444.360	0.6824 (0.4438, 1.0493), 0.0817	0.9401 (0.5782, 1.5287), 0.8033
>444.360	1 (-)	1 (-)
Cortisol ≤322.644 (nmol/L)	**0.7160 (0.5184, 0.9890), 0.0426**	0.7588 (0.5271, 1.0925), 0.1378
Cortisol ≤444.360 (nmol/L)	0.6257 (0.4448, 0.8801), **0.0071**	0.8000 (0.5445, 1.1752), 0.2555
Log(Cortisol)	1.2265 (0.9386, 1.6027), 0.1347	1.1110 (0.8362, 1.4762), 0.4679

Kaplan Meier curves for cortisol censoring at 1 year unadjusted and adjusted for independent prognostic markers are displayed in Figure 10-24 to Figure 10-26.

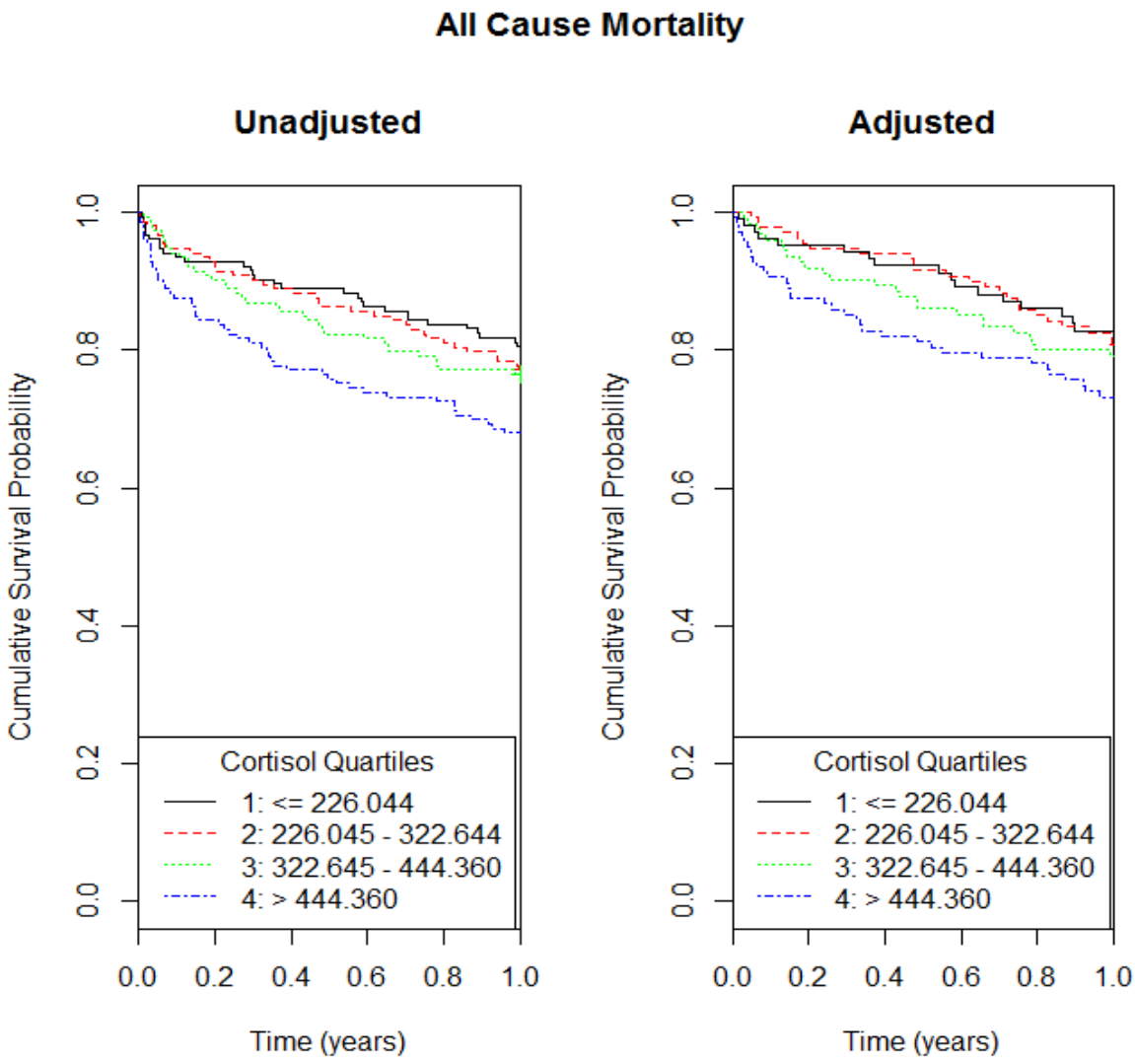


Figure 10-24. Unadjusted and adjusted Kaplan-Meier curves for all-cause mortality at 1 year after hospital admission according to quartiles of plasma cortisol at baseline

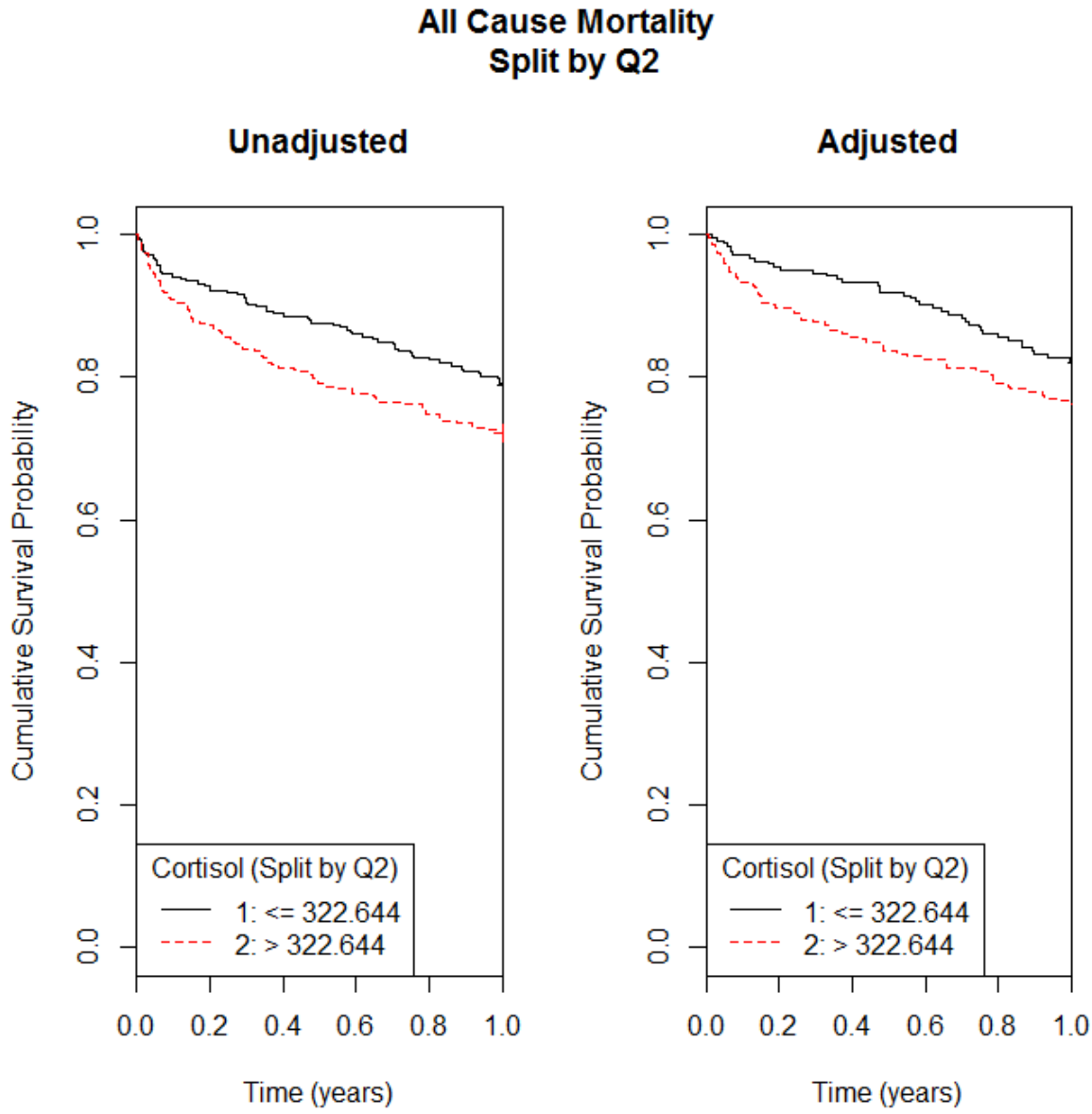


Figure 10-25. Unadjusted and adjusted Kaplan-Meier curves for all-cause mortality at 1 year after hospital admission according to plasma cortisol (dichotomised according to median, Q2) at baseline

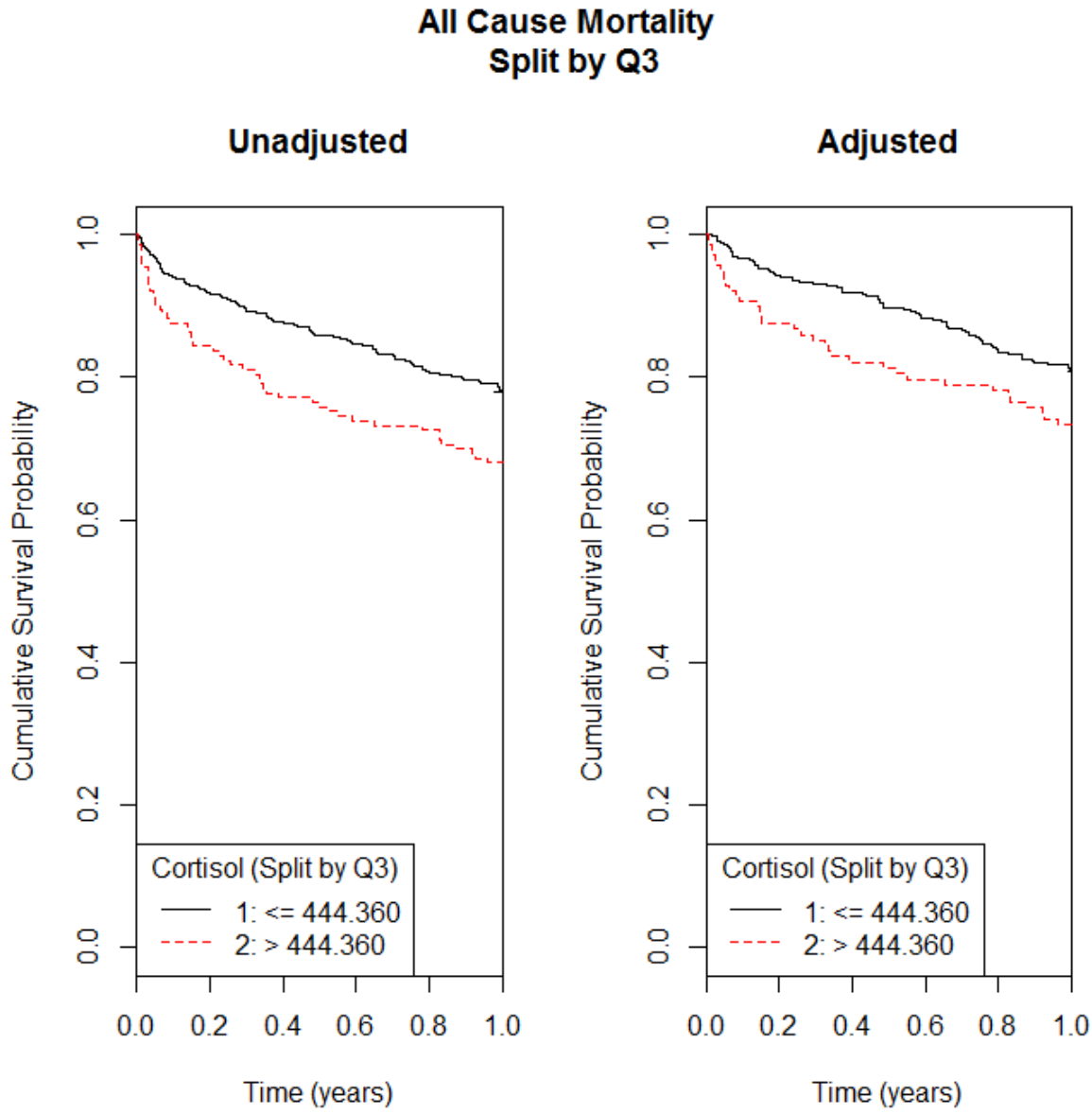


Figure 10-26. Unadjusted and adjusted Kaplan-Meier curves for all-cause mortality at 1 year after hospital admission according to plasma cortisol (dichotomised according to 75th percentile, Q3) at baseline

10.4 Discussion

10.4.1 RAAS mediators and prognosis

To my knowledge this is the first study to examine the prognostic value of PRC in a wide spectrum of patients with acute decompensated HF. The prognostic importance of renin was previously examined in patients with chronic HF with LVSD (353) (354) (403). In these studies plasma renin, measured either as activity or concentration, was positively associated with increased risk of mortality. In a similar fashion, higher PRC showed prognostic value in patients with decompensated HF over-and-above other independent prognostic markers in the current study.

What are the potential links which account for the association of PRC with all-cause mortality in patients with decompensated HF? Firstly, high renin concentrations may represent a marker of worse cardiac and/or renal disease. Indeed, PRC was associated with lower blood pressure, indexes of LV remodeling and markers of renal function in patients during hospital admission. However, renin retained its prognostic significance when adjusted for markers of haemodynamic status and renal function in most of the models, indicating that other factors likely to contribute additionally to its prognostic importance. A potential alternative link might be the deleterious effects of renin on the cardiovascular system through RAAS activation in the long term; as previously discussed, aldosterone levels in patients with worsening HF were higher in patients with higher PRC despite treatment with a RAAS inhibitor, indicating that the secretion of the down-stream components of RAAS continues to be primarily driven by renin despite the treatment with an ACE inhibitor/ARB. Moreover, the reactive rise of renin due to RAAS may overcome RAAS inhibition leading to further disease progression. On the other hand, higher renin concentrations might be of additional

physiological importance, as there is growing evidence that renin exerts direct inflammatory and fibrotic effects independent of angiotensin II through prorenin/renin receptors (404) (405)

Apart from the potential direct and indirect effects of renin on disease progression and clinical outcomes, other factors might also account for the observed association between renin and all-cause mortality. Higher PRC levels may reflect the inhibition of RAAS with an ACE inhibitor/ARB or an aldosterone blocker. Indeed, as was shown previously PRC was higher in patients taking a RAAS inhibitor prior to admission. On the other hand, prior treatment with a RAAS inhibitor reflects history of established cardiovascular disease and that may have further contributed to worse outcomes seen in patients with higher renin levels. However, it is likely that a number of patients with a history of HF, especially those with advanced HF, were not treated with a RAAS inhibitor prior to hospital admission due to haemodynamic instability or kidney dysfunction. PRC rise in these patients due to disease progression might not be as prominent as in patients treated with RAAS inhibitors; nevertheless, it is generally accepted that patients with advanced HF not taking RAAS inhibitors have worse prognosis and higher short-term mortality. That might account for the difference in the prognostic value of PRC in 12 months after hospital admission and in the overall follow-up in the current study.

Finally, other unmeasured variates may contribute to the increased risk of death in patients with higher PRC during hospital admission in the current study. One of the principal regulators of renin secretion is the SNS activation. Plasma norepinephrine has been associated with worse outcomes in patients with severe HF (384). Thus, greater SNS activity promotes RAAS activation and may contribute to the higher mortality risk in patients with higher PRC and worsening HF.

In contrast to PRC, plasma aldosterone was not associated with prognosis in the current study. In patients with congestive HF not taking an ACE inhibitor or ARB aldosterone levels were associated with worse medium-term prognosis in CONSENSUS trial (55). Moreover, in patients with decompensated HF in the EVEREST trial, aldosterone was univariately associated with all-cause mortality (340). Aldosterone was also associated with all-cause mortality after adjustment for established prognostic factors in that study (Prof Faiez Zannad, personal communication, November 2012). However, it should be mentioned that approximately half of these patients were taking an aldosterone blocker; that is likely to affect the association between aldosterone levels and survival, as aldosterone blockers increase aldosterone levels and their prescription is targeted to patients with severe HF and hence worse prognosis. Alternatively, as previously discussed different methods were employed for aldosterone measurement and that may contribute to the differences in aldosterone levels and their associations with mortality between the current and the EVEREST study. Aldosterone levels were measured in the current study by LCMS, which has been recognised for its specificity over immunoassays, specifically with regards to aldosterone measurements (323). Overall, in contrast to previous studies aldosterone levels in the current study were not found to be a prognostic indicator in patients with decompensated HF.

Finally, the aldosterone to renin ratio was univariately associated with outcomes; however, that was not in a stepwise fashion and although the mortality risk was higher in patients with the highest aldosterone to renin ratio quartile, the worst prognosis was not seen in patients in lowest quartile. As discussed in chapter 6, the aldosterone to renin ratio is principally driven by renin in patients with HF and that is likely to account for the better outcomes in patients with the higher ratio. Nevertheless, treatment with RAAS inhibitors exerts discordant effects on RAAS mediators with variable effects on the aldosterone to renin ratio which potentially reduce its prognostic significance. Thus, despite the stronger association with features of HF

severity in patients not taking a RAAS inhibitor, as previously shown in chapter 7, the aldosterone to renin ratio is not as discriminating as PRC in identifying HF patients with worse prognosis following RAAS inhibition.

10.4.2 Glucocorticoid levels and survival

11-deoxycortisol levels were not associated with all-cause mortality in the current cohort. That is not entirely surprising given that 11-deoxycortisol is an intermediate corticosteroid with weak mineralocorticoid activity. In addition, the 11-deoxycortisol to cortisol ratio, an index of 11beta-hydroxylase activity, was not associated with increased risk of mortality in the overall population. The association of 11-deoxycortisol to cortisol ratio with prognostic markers of HF but no with outcomes indicates that 11beta-hydroxylase was up-regulated in patients with features of severe HF, however, the greater activity of this enzyme was not related with mortality. Nevertheless, as all-cause mortality was the only end point in the current study, an impact of greater 11beta-hydroxylase activity on HF progression and hospitalisation or combined end points cannot be excluded.

Cortisol was associated with all-cause mortality at 1year after hospitalisation in univariate but not with long term prognosis in the current study; 6 months after hospital admission the survival curves of cortisol quartiles were almost parallel and the hazards were proportional. These findings indicate that cortisol levels in patients with decompensated HF represent mainly the HF severity and comorbidity during hospital admission with decompensated HF. In other words cortisol levels are likely to reflect the stress of acute illness but do not confer prognostic importance in the long term. That is in accordance with the finding that the risk of death is higher early after a hospitalisation for HF and is affected by the length of stay which is influenced mainly by HF severity and comorbidity (346). On the other hand, whether cortisol is associated with long term prognosis in patients with stable HF has not

been examined in this thesis; previous studies reported on the independent prognostic significance of cortisol in patients with chronic HF, suggesting a patho-physiological role of glucocorticoids in HF progression through the activation of MRs (72) (73). The discrepancy in the findings between the current and the above mentioned studies indicate that the prognostic cortisol role might be different depending on HF stage.

Finally, the effect of diurnal rhythm on glucocorticoid levels, should be taken into account in the interpretation of the findings in the current study. In the studies with the healthy subjects, there was a significant decline in cortisol levels from 8am to 12pm during ambulatory blood sampling. Blood samples were collected between 8-11am in patients with HF during hospital admission; thus, the circadian rhythm is likely to have a prominent effect on the association between glucocorticoids and outcomes and potentially limit the prognostic power of cortisol. Future studies examining the prognostic value of cortisol with collection of blood samples within a narrower time frame in the morning or evening will give further insights into the prognostic value of corticosteroids

In conclusion, PRC showed an independent prognostic value in a broad spectrum of patients with decompensated HF over and above variables that represent distinct pathophysiological pathways, such as age, SBP, urea, history of COPD, haemoglobin, troponin and log(BNP). In contrast, cortisol although showed a medium-term prognostic value, it did not remain significant following adjustment for similar factors. Renin is the principal regulator of RAAS and might contribute directly or indirectly in HF development and progression despite treatment with a RAAS inhibitor. Moreover, renin is linked with other pathways, such as SNS, playing a pivotal which in HF progression. Measurement of renin in patients

with decompensated HF may help identifying those who need further therapeutic measures. Future studies will clarify if such a strategy is translated into clinical benefit.

11. Chapter Eleven - Associations of *CYP11B2* polymorphisms with mineralo- and gluco-corticoid secretion and prognosis in patients with HF

11.1 Introduction

Higher levels of cortisol and aldosterone have been found to be associated with worse prognosis in HF (62, 63). The final step in cortisol synthesis from 11-deoxycortisol in adrenal ZF is catalysed by the enzyme 11beta-hydroxylase, which is encoded by the *CYP11B1* gene. Aldosterone synthase, catalyses the final steps of aldosterone production in ZG and is encoded by *CYP11B2*. A common polymorphism (-344C/T) in the promoter region of *CYP11B2* has been shown to be associated with aldosterone levels and blood pressure (298) (301). The -344T allele, moreover, has been consistently associated with a higher 11-deoxycortisol to cortisol ratio in urine and plasma, which represent an index of impaired 11beta-hydroxylase efficiency (299) (311).

In patients with HF, the *CYP11B2* -344TT genotype has been found to be linked with better event-free survival in African-Americans (315). In addition, the same genotype has been shown to be associated with better outcomes in patients of European ethnicity following MI (317). However, the prognostic significance of *CYP11B2* polymorphisms in patients with HF of Caucasian origin remains unknown.

The aim of this chapter is to explore the potential impact of *CYP11B2* -344 T/C polymorphism and IC on mineralo- and gluco- corticoid levels and the 11-deoxycortisol to cortisol and aldosterone to PRC ratio in patients of Caucasian origin with decompensated HF. In this chapter, I also examine the prognostic significance of *CYP11B2* polymorphisms in this cohort of patients.

11.2 Methods

11.2.1 Study design and laboratory measurements

The study design and laboratory measurements in the overall hospitalised cohort were described in sections 2.3.1 & 2.3.2.

11.2.2 DNA extraction and genotyping

The methodology of DNA extraction and genotyping of *CYP11B2* -344T/C and IC polymorphisms was described in section 2.3.3. Genotyping was performed in 699 (96.8%) patients for the *CYP11B2* -344 T/C polymorphism and in 696 (96.3%) patients for the *CYP11B2* IC.

11.2.3 Follow-up

The primary outcome in this study was all-cause mortality, defined as death in and out of the hospital from any cause. Survival was defined as the period from the enrollment in the study during hospital admission until the time of death or the censor date on 31st of August 2010.

11.2.4 Statistical analysis

All patient characteristics were expressed as median (IQR) for continuous and as absolute number (percentage) for categorical variables. The comparisons among the different genotype groups were carried out by the Mann-Whitney and Kruskal-Wallis test for continuous variables as appropriate and by the χ^2 test for categorical variables. For the outcome analyses, Kaplan-Meier event-free (time to death) survival curves were constructed for each of the *CYP11B2* -344T/C and IC genotypes in the overall hospitalised cohort. The log-rank test was used for the comparison of the survival curves by genotypes. Cox regression analysis was employed to calculate the HR of all-cause mortality over time according to a reference genotype. In addition, given the data from previous studies showing

that homozygotes for the -344T allele had better prognosis compared to heterozygotes or homozygotes for the -344C allele, CC and TC patients were pooled in one group (CC + TC) and compared with TT patients in a separate Kaplan-Meier survival analysis. A p-value <0.05 was considered significant for all the analyses.

11.3 Results

11.3.1 Genotype distribution

The clinical characteristics of the 722 patients enrolled in the study during the hospital admission were presented in section 4.3.1. The majority of patients (n=714; 98.9%) were of Caucasian origin and the rest of the patients were of South Asian and African-Caribbean origin. Genotyping for the *CYP11B2* -344 T/C polymorphism classified 154 (22%) patients as homozygous for the C allele (CC), 345 (49%) as heterozygous (CT) and 200 (29%) as homozygous for the T allele (TT). The above genotype frequencies (% CC/CT/TT = 22%/49%/29%) were consistent with the Hardy-Weinberg equilibrium. Moreover, they were very similar to *CYP11B2* -344T/C genotype frequencies identified in the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) (% CC/CT/TT = 23%/50%/27%) (Prof Ian Ford, personal communication, December 2011). The PROSPER study recruited patients of Caucasian origin in Scotland, Ireland and the Netherlands, who had or were at high risk for vascular disease (406). In contrast, the genotype frequencies in my study differed markedly from the frequencies of -344T/C polymorphism reported in a cohort of African Americans from the GRAHF study, a genetic sub-study of the A-HeFT study (% CC/CT/TT = 6%/32%/62%). That is expected as the 344T allele has been reported to be more prevalent in African-Americans (301).

Genotyping for the *CYP11B2* IC revealed 137 (20%) homozygotes for the conversion allele (CON), 321 (46%) heterozygotes (HTZ) and 238 (34%) homozygotes for the wild-type allele (WT). The above genotype distribution was consistent with Hardy-Weinberg equilibrium.

11.3.2 Patient characteristics according to *CYP11B2* -344T/C genotypes

The clinical characteristics during hospital admission in patients of the overall cohort according to *CYP11B2* -344T/C genotypes (CC/TC/TT) are presented in Table 11-1. Patients with the CC genotype were more likely to have a history of previous MI compared to patients with the TT and TC genotypes. Serum sodium levels were lower in patients with CC and TC genotypes and higher in patients with TT genotype. Homozygotes for the C allele were more often in NYHA class IV and less often in NYHA class II compared to heterozygotes and homozygotes for the T allele, although the above differences failed to reach statistical significance.

With regards to plasma glucocorticoid levels, 11-deoxycortisol levels and 11-deoxycortisol to cortisol ratio increased in a stepwise fashion according to the increasing number of T alleles. Indeed, TT patients were more likely to have higher 11-deoxycortisol levels and 11-deoxycortisol to cortisol ratio and CC patients were more likely to have lower 11-deoxycortisol levels and 11-deoxycortisol to cortisol ratio, with TC patients being intermediate. No differences in cortisol levels were detected among the three genotype subgroups.

Table 11-1. Patient characteristics during hospital admission according to *CYP11B2* -344T/C genotypes

Variable	CC (n=154)	TC (n=345)	TT (n=200)	p-value†
Age (years)	75 (68 – 82)	73 (67 – 80)	74 (69 – 82)	0.309
Female gender	66 (42.9)	157 (45.5)	99 (49.5)	0.442
NYHA class				
II	34 (22.1)	75 (21.7)	61 (30.5)	0.054
III	93 (60.4)	210 (60.9)	115 (57.5)	0.731
IV	27 (17.5)	60 (17.4)	24 (12)	0.206
Medical history				
HF	71 (46. 1)	143 (41.5)	91 (45.5)	0.513
MI	81 (52.6)	140 (40.6)	88 (44)	**0.044**
Angina	90 (58.4)	184 (53.3)	109 (54.5)	0.568
Diabetes mellitus	45 (29.2)	109 (31.6)	65 (32.5)	0.796
Hypertension	99 (64.3)	235 (68.1)	129 (64.5)	0.584
AF	76 (49.4)	191 (55.4)	109 (54.5)	0.448
CVA/TIA	37 (24)	75 (21.7)	39 (19.5)	0.589
Physiological measurements				
BMI (kg/m^2)	27.3 (23.6 – 32.6)	28.2 (24.4 – 33.2)	27.8 (23.7 – 33.4)	0.384
Pulse rate (bpm)	89 (72 – 108)	84 (72 – 106)	88 (72 – 104)	0.636
SBP (mmHg)	129 (114 – 144)	135 (116 – 152)	135 (115 – 154)	0.064
DBP (mmHg)	74 (61 – 85)	76 (64 – 89)	75 (60 – 87)	0.351

Variable	CC (n=154)	TC (n=345)	TT (n=200)	p-value†
ECG rhythm				
SR	89 (57.8)	186 (53.9)	110 (55)	0.723
AF	59 (38.3)	142 (41.2)	84 (42)	0.766
Signs of fluid congestion				
Elevated JVP	110 (78.6)	236 (77.4)	149 (82.8)	0.359
Peripheral oedema	113 (73.4)	260 (75.4)	150 (75)	0.892
Echocardiogram measurements				
LVEDD (cm)	5.2 (4.6 – 5.9)	5.2 (4.7 – 6.0)	5.2 (4.7 – 6.0)	0.774
Dilated left ventricle	38 (35.2)	92 (37.1)	57 (40.7)	0.647
LVH	52 (49.1)	110 (44.5)	58 (41.4)	0.491
LVSD	74 (68.5)	164 (66.1)	95 (67.9)	0.887
Laboratory measurements (blood)				
BNP (pg/ml)	764.5 (400 – 1896)	874 (371 – 1826)	944 (412 – 1798)	0.906
Troponin I ≥ 0.04 (µg/L)*	70 (51.85)	164 (57.1)	86 (53.4)	0.540
Sodium (mmol/L)	138 (135 – 140)	138 (135 – 140)	139 (136 – 141)	**0.032**
Potassium (mmol/L)	4.2 (3.8 – 4.6)	4.2 (3.8 – 4.5)	4.2 (3.9 – 4.5)	0.962
Urea (mmol/L)	8.6 (6.0 – 11.7)	8.7 (6.5 – 11.9)	8.4 (6.3 – 12.0)	0.709
Creatinine (µmol/L)	108 (85 – 137)	108 (86 – 136)	105 (83 – 137)	0.907
eGFR (ml/min/1.73m^2)	56 (41 – 60)	56 (42 – 60)	59 (40 – 60)	0.873
eGFR <60ml/min/1.73m^2	86 (55.8)	196 (56.8)	105 (52.5)	0.615

Variable	CC (n=154)	TC (n=345)	TT (n=200)	p-value†
Cholesterol (total) (mmol/L)	3.7 (3.0 – 4.7)	3.7 (3.1 – 4.6)	3.7 (3.1 – 4.6)	0.951
HDL (mmol/L)	0.9 (0.8 – 1.3)	1.0 (0.8 – 1.3)	1.0 (0.8 – 1.3)	0.705
CRP (mg/L)	13 (6 – 25)	13 (5 – 35)	13 (6 – 27)	0.878
TSH (mIU/L	1.9 (1.2 – 3.0)	1.7 (1.0 – 2.7)	1.7 (1.0 – 2.9)	0.202
Cortisol (nmo/L)	306.8 (213.3 – 409.0)	341.1 (245.9 – 443.5)	309.4 (222.3 – 233.6)	0.118
11-deoxycortisol (pmol/L)	393.6 (206.2 – 793.4)	495 (291 – 925.0)	571.4 (308.2 – 978.5)	**0.019**
11-deoxycortisol/cortisol (10^{-3})	1.46 (0.73 – 2.53)	1.58 (0.94 – 2.92)	1.84 (1.12 – 2.96)	**0.034**
Aldosterone (pmol/L)	68.9 (30.1 – 164.0)	74.8 (37.3 – 157.0)	70.9 (27.5 – 140.4)	0.585
PRC (mIU/L)	48.7 (15.2 – 184.0)	48.4 (15.1 – 158.2)	38.3 (11.0 – 181.0)	0.477
Aldosterone/PRC	1.54 (0.27 – 5.13)	1.61 (0.32 – 4.84)	1.33 (0.24 – 5.24)	0.999
Haemoglobin (g/dl)	12.2 (11.0 – 13.8)	12.0 (10.5 – 13.5)	12.2 (10.5 – 13.5)	0.460
Cardiovascular medication prior to admission				
Diuretic	102 (66.2)	236 (68.4)	142 (71)	0.625
ACE inhibitor	74 (48.1)	169 (49)	102 (51)	0.844
ACE inhibitor or ARB	84 (54.6)	204 (59.1)	128 (64)	0.195
Beta-blocker	74 (48.1)	160 (46.4)	99(49.5)	0.775
Aldosterone antagonist	9 (5.8)	23 (6.7)	13 (6.5)	0.941
Digoxin	22 (14.3)	55 (15.9)	35 (17.5)	0.715
Anti-arrhythmic	4 (2.6)	17 (4.9)	7 (3.5)	0.430
Aspirin	82 (53.3)	188 (54.5)	103 (51.5)	0.796

Variable	CC (n=154)	TC (n=345)	TT (n=200)	p-value†
Statin	106 (68.8)	223 (64.6)	128 (64)	0.588
Steroid tablets	5 (3.3)	12 (3.5)	9 (4.5)	0.782

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).

† Kruskal-Wallis test was used for continuous variables and χ test for categorical variables.

*measured at WIG and GRI

Looking further at the inter-group differences in 11-deoxycortisol levels and 11-deoxycortisol to cortisol ratio, 11-deoxycortisol was significantly higher in patients with the TT genotype compared to patients with the CC genotype (Figure 11-1 below). Similarly, 11-deoxycortisol levels remained significantly higher in TC patients compared to CC patients. In contrast, TT patients had numerically but not statistically higher 11-deoxycortisol compared to TC patients.

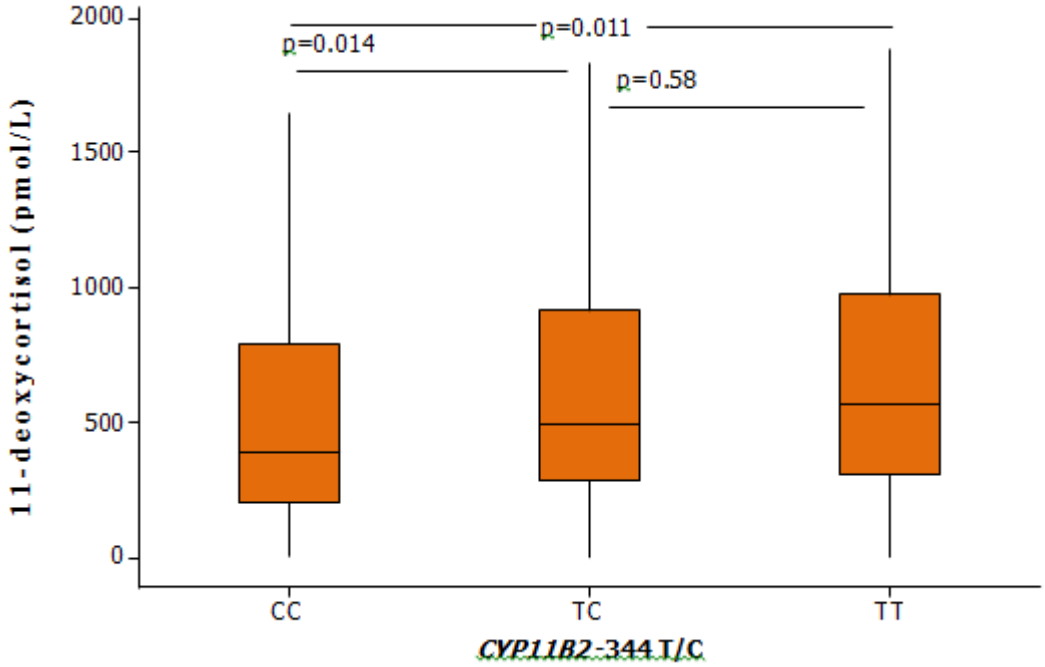


Figure 11-1. 11-deoxycortisol levels in all subjects of the hospitalised cohort according to *CYP11B2* -344T/C genotypes. Inter-group comparisons performed by Mann-Whitney nonparametric testing

Similar to 11-deoxycortisol, the 11-deoxycortisol to cortisol ratio was found to be significantly higher in patients with TT genotype compared with patients with CC genotype (Figure 11-2 below). TC patients were more likely to have higher 11-deoxycortisol to cortisol ratio compared to CC patients, and TT patients were more likely to have higher 11-deoxycortisol to cortisol ratio compared to TC patients, but these differences failed to reach statistical significance.

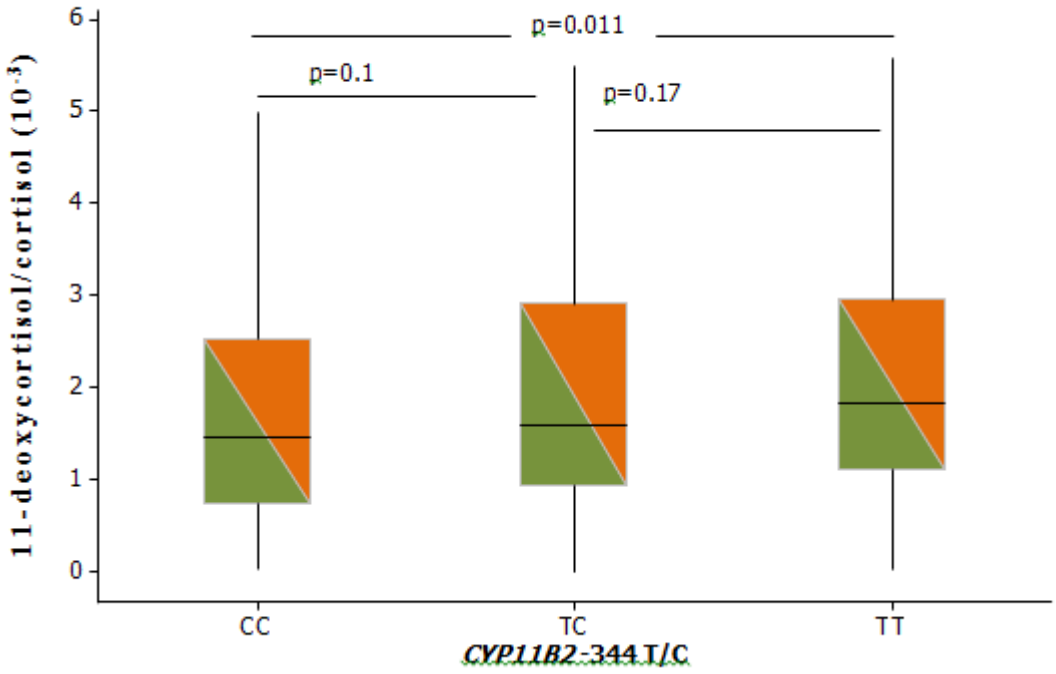


Figure 11-2. 11-deoxycortisol to cortisol ratio in the hospitalised cohort according to *CYP11B2* -344T/C genotypes. Inter-group comparisons performed by Mann-Whitney nonparametric testing

In contrast to 11-deoxycortisol levels and the 11-deoxycortisol to cortisol ratio, plasma aldosterone and renin concentrations and the aldosterone to PRC ratio were not different among the *CYP11B2* -344T/C genotype subgroups. Given the impact of background therapy

with a RAAS inhibitor on PRC and aldosterone levels (section 4.3.2.4), I explored further if potential associations of *CYP11B2* -344T/C polymorphism with aldosterone and the aldosterone to PRC ratio (or other clinical characteristics) exist in the absence of background treatment with an ACE inhibitor/ARB or aldosterone antagonist (Table 11-2). Aldosterone, PRC and the aldosterone to PRC ratio were not different among the genotype groups. In contrast to the overall hospitalised cohort, there were no differences in 11-deoxycortisol levels and the 11-deoxycortisol to cortisol ratio among patients with different genotypes, likely due to the markedly smaller study cohort

Table 11-2. Clinical characteristics during hospital in patients not taking an ACE inhibitor/ARB or aldosterone blocker according to *CYP11B2* -344T/C genotypes

Variable	CC (n=66)	TC (n=136)	TT (n=68)	p-value†
Age (years)	75 (68 – 82)	74 (67 – 80)	77 (66 – 83)	0.861
Female gender	32 (48.5)	67 (49.3)	44 (64.7)	0.080
NYHA class				
II	15 (22.7)	35 (25.7)	20 (29.4)	0.676
III	41 (62.1)	80 (58.8)	39 (57.4)	0.845
IV	10 (15.2)	21 (15.4)	9 (13.2)	0.913
Medical history				
HF	23 (34.9)	30 (22.1)	15 (22.1)	0.115
MI	29 (43.9)	39 (28.7)	19 (27.9)	0.064
Angina	31 (47)	51 (37.5)	33 (48.5)	0.230
Diabetes mellitus	14 (21.2)	21 (15.4)	11 (16.2)	0.579
Hypertension	40 (60.6)	78 (57.4)	38 (55.9)	0.849
AF	27 (40.9)	73 (53.7)	38 (55.9)	0.155
CVA/TIA	17 (25.7)	25 (18.4)	12 (17.7)	0.401
Physiological measurements				
BMI (kg/m^2)	26.5 (22.7 – 31.2)	27.4 (22.6 – 32.4)	25.7 (22.3 – 31.7)	0.686
Pulse rate (bpm)	93 (74 – 113)	96 (77 – 110)	94 (77 – 110)	0.997
SBP (mmHg)	131 (117 – 152)	138 (122 – 152)	139 (120 – 156)	0.378

Variable	CC (n=66)	TC (n=136)	TT (n=68)	p-value†
DBP (mmHg)	80 (62 – 93)	81 (68 – 92)	78 (65 – 94)	0.802
ECG rhythm				
SR	45 (68.2)	73 (53.7)	33 (48.5)	0.055
AF	21 (31.8)	57 (41.9)	32 (47.1)	0.185
Signs of fluid congestion				
Elevated JVP	48 (82.8)	89 (73)	50 (83.3)	0.168
Peripheral oedema	43 (65.2)	102 (75)	49 (72.1)	0.344
Echocardiogram measurements				
LVEDD (cm)	5.1 (4.6 – 5.8)	5.0 (4.6 – 5.7)	5.1 (4.5 – 5.9)	0.686
Dilated left ventricle	1938 (35.2)	34 (30.9)	22 (40)	0.503
LVH	27 (50.9)	46 (41.8)	22 (40)	0.451
LVSD	34 (63)	74 (67.3)	41 (74.6)	0.419
Laboratory measurements (blood)				
BNP (pg/ml)	815 (400 – 2005)	852 (355 – 1599)	976 (495 – 2143)	0.350
Troponin I ≥ 0.04 (µg/L)*	30 (53.6)	65 (57.0)	32 (59.3)	0.830
Sodium (mmol/L)	138 (135 – 140)	138 (135 – 140)	139 (135 – 141)	0.545
Potassium (mmol/L)	4.1 (3.7 – 4.6)	4.2 (3.9 – 4.5)	4.1 (3.8 – 4.5)	0.772
Urea (mmol/L)	7.2 (5.7 – 11.0)	8.1 (6.0 – 10.9)	7.9 (6.4 – 10.0)	0.709
Creatinine (µmol/L)	101 (83 – 129)	100 (82 – 126)	96 (80 – 123)	0.675
eGFR (ml/min/1.73m^2)	60 (47 – 60)	59 (43 – 60)	60 (45 – 60)	0.801

Variable	CC (n=66)	TC (n=136)	TT (n=68)	p-value†
eGFR <60ml/min/1.73m^2	33 (50)	72 (53)	32 (47.1)	0.724
Cholesterol (total) (mmol/L)	4.3 (3.1 – 5.0)	4.0 (3.5 – 4.9)	4.1 (3.4 – 4.9)	0.960
HDL (mmol/L)	1.1 (0.8 – 1.4)	1.0 (0.8 – 1.3)	1.0 (0.8 – 1.4)	0.738
CRP (mg/L)	11 (5 – 22)	14 (6 – 39)	15 (7 – 32)	0.273
TSH (mIU/L	1.8 (1.4 – 2.06	1.8 (1.2 – 2.8)	1.7 (1.0 – 2.9)	0.746
Cortisol (nmo/L)	306.1 (219.7 – 443.8)	358.0 (261.3 – 437.2)	332.9 (222.5 – 478.9)	0.335
11-deoxycortisol (pmol/L)	542.1 (283.2 – 1190)	498.2 (319.9 – 893.5)	497.9 (241.2 – 964.0)	0.894
11-deoxycortisol/cortisol (10^{-3})	1.80 (1.23 – 2.99)	1.57 (1.02 – 2.78)	1.67 (0.93 – 3.18)	0.562
Aldosterone (pmol/L)	87.7 (50.2 – 226.6)	77.6 (41.3 – 158.6)	89.9 (40.1 – 184.7)	0.576
PRC (mIU/L)	30.4 (10.4 – 78.2)	29.3 (9.3 – 71.5)	19.4 (6.3 – 76.3)	0.545
Aldosterone/PRC	3.41 (1.39 – 7.71)	3.03 (1.10 – 6.55)	3.40 (0.85 – 12.36)	0.599
Haemoglobin (g/dl)	12.7 (11.3 – 14.2)	12.5 (10.7 – 14.0)	12.4 (11.1 – 13.9)	0.709
Cardiovascular medication prior to admission				
Diuretic	34 (51.5)	67 (49.3)	37 (54.4)	0.784
Beta-blocker	22 (33.3)	49 (36)	26 (38.2)	0.839
Digoxin	7 (10.6)	15 (11)	10 (14.7)	0.699
Anti-arrhythmic	2 (3)	5 (3.7)	3 (4.4)	0.914
Aspirin	34 (51.5)	66 (48.5)	27 (39.7)	0.346
Statin	38 (57.6)	61 (44.9)	30 (44.1)	0.185

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).
† Kruskal-Wallis test was used for continuous variables and χ^2 test for categorical variables.
*measured at WIG and GRI

11.3.3 Patient characteristics according to *CYP11B2* IC genotypes

The clinical characteristics during the hospital admission in patients of the overall cohort according to *CYP11B2* IC genotypes (Con/Htz/Wt) are presented in Table 11-3. Patients with the Con genotype were more likely to be in NYHA functional class II and less likely to be in NYHA functional class IV compared to patients with the Htz and Wt genotypes. There was also a trend for higher sodium and lower prevalence of elevated troponin in Con patients compared to Htz and Wt patients.

With regards to plasma glucocorticoid levels, a trend for higher 11-deoxycortisol and 11-deoxycortisol to cortisol ratio was present in patients with Con genotype compared to patients with Htz and Wt genotypes, however, the differences were not significant.

Similar to the -344T/C genotypes in the hospitalised cohort, no differences in plasma aldosterone and renin concentrations and the aldosterone to PRC ratio were seen among the *CYP11B2* IC genotypes.

Table 11-3. Patient characteristics of the hospitalised cohort according to *CYP11B2* intron 2 genotype subgroups

Variable	Con (n=137)	Htz (n=321)	Wt (n=238)	p-value†
Age (years)	74 (68 - 81.5)	74 (68 - 80)	74 (67 - 80.3)	0.666
Female gender	66 (48.2)	145 (45.2)	110 (46.2)	0.839
NYHA class				
II	46 (33.6)	69 (21.5)	54 (22.7)	**0.017**
III	78 (56.9)	195 (60.7)	143 (60.1)	0.742
IV	13 (9.5)	57 (17.8)	41 (17.2)	0.069
Medical history				
HF	58 (42.3)	136 (42.4)	109 (45.8)	0.686
MI	57 (41.6)	139 (43.3)	111 (46.6)	0.591
Angina	71 (51.8)	178 (55.5)	133 (55.9)	0.721
Diabetes mellitus	40 (29.2)	107 (33.3)	72 (30.3)	0.604
Hypertension	82 (59.9)	223 (69.5)	155 (65.1)	0.128
AF	75 (54.7)	174 (54.2)	123 (51.7)	0.792
CVA/TIA	22 (16.1)	74 (23.1)	54 (22.7)	0.217
Physiological measurements				
BMI (kg/m^2)	27.8 (23.7 - 32.8)	28.1 (23.9 - 33.1)	27.9 (24.1 - 33.1)	0.947
Pulse rate (bpm)	88 (70 - 104)	85 (72 - 107)	88 (72 - 108)	0.989
SBP (mmHg)	134 (114 – 155)	135 (115.5 - 152)	130 (115 - 150)	0.261
DBP (mmHg)	75 (60 - 89)	75 (63 - 88)	74 (62 - 88)	0.802

Variable	Con (n=137)	Htz (n=321)	Wt (n=238)	p-value†
ECG rhythm				
SR	77 (56.2)	170 (53)	139 (58.4)	0.432
AF	55 (40.2)	137 (42.7)	89 (37.4)	0.452
Signs of fluid congestion				
Elevated JVP	102 (81)	217 (77)	173 (80.8)	0.486
Peripheral oedema	100 (73)	242 (75.4)	178 (74.5)	0.864
Echocardiogram measurements				
LVEDD (cm)	5.25 (4.5 - 6.1)	5.18 (4.73 - 5.93)	5.2 (4.6 - 5.9)	0.751
Dilated left ventricle	40 (42.1)	85 (37)	62 (36.9)	0.647
LVH	38 (40)	102 (44.5)	78 (47)	0.550
LVSD	63 (66.3)	155 (67.4)	114 (67.9)	0.968
Laboratory measurements (blood)				
BNP (pg/ml)	897 (403 - 1790)	964 (401 - 1929)	762 (362 - 1804)	0.426
Troponin I ≥ 0.04 (µg/L)*	52 (47.3)	163 (59.7)	104 (52.3)	0.058
Sodium (mmol/L)	139 (135 – 141)	138 (135.5 - 140)	138 (135 - 140)	0.074
Potassium (mmol/L)	4.1 (3.9 - 4.5)	4.2 (3.8 – 4.6)	4.2 (3.8 - 4.5)	0.349
Urea (mmol/L)	8.4 (6.5 - 11.5)	8.7 (6.5 - 12)	8.7 (6 - 11.7)	0.593
Creatinine (µmol/L)	104 (83 - 135.5)	109 (87 - 136.5)	104 (83 - 136.3)	0.390
eGFR (ml/min/1.73m^2)	59 (42 - 60)	55 (41 - 60)	56 (43 - 60)	0.374
eGFR <60ml/min/1.73m^2	71 (51.8)	186 (57.9)	128 (53.8)	0.407

Variable	Con (n=137)	Htz (n=321)	Wt (n=238)	p-value†
Cholesterol (total) (mmol/L)	4 (3.3 – 4.6)	3.7 (3.1 – 4.6)	3.7 (3 - 4.5)	0.538
HDL (mmol/L)	1 (0.8 – 1.4)	1 (0.8 - 1.3)	1 (0.8 - 1.3)	0.573
CRP (mg/L)	12 (6.1 - 27.8)	14 (5.7 - 34)	12 (5.1 - 25.5)	0.508
TSH (mIU/L	1.7 (1.1 - 2.9)	1.6 (1 - 2.7)	1.8 (1.1 - 2.7)	0.612
Cortisol (nmo/L)	317.1 (224.5 - 450.2)	327.1 (229.8 - 441.7)	320.4 (219.8 - 444.1)	0.838
11-deoxycortisol (pmol/L)	533.9 (267.5 - 982.3)	482.8 (300 - 915)	473.1 (233 - 933)	0.335
11-deoxycortisol/cortisol (10^{-3})	1.84 (1.12 - 2.92)	1.62 (0.94 - 2.95)	1.56 (0.93 - 2.72)	0.238
Aldosterone (pmol/L)	73.8 (37.8 – 137.3)	74.9 (32.7 - 158.1)	70.5 (30.8 - 157.4)	0.992
PRC (mIU/L)	42.8 (12 – 205)	49.3 (15.3 - 154.4)	47.3 (13.3 - 185.4)	0.870
Aldosterone/PRC	1.52 (0.46 - 5.22)	1.55 (0.32 - 4.62)	1.55 (0.25 - 5.84)	0.960
Haemoglobin (g/dl)	12.3 (10.9 - 13.8)	12 (10.9 - 13.5)	12.1 (10.4- 13.7)	0.614
Cardiovascular medication prior to admission				
Diuretic	95 (69.3)	219 (68.2)	163 (68.5)	0.972
ACE inhibitor	61 (44.5)	160 (49.8)	122 (51.2)	0.437
ACE inhibitor or ARB	80 (58.4)	191 (59.5)	143 (60.1)	0.950
Beta blocker	68 (49.6)	154 (48)	111 (46.6)	0.853
Aldosterone antagonist	10 (7.3)	20 (6.2)	16 (6.7)	0.912
Digoxin	21 (15.3)	53 (16.5)	37 (15.6)	0.931
Anti-arrhythmic	1 (0.73)	16 (4.98)	10 (4.2)	0.092
Aspirin	66 (48.2)	178 (55.5)	128 (53.8)	0.357

Variable	Con (n=137)	Htz (n=321)	Wt (n=238)	p-value†
Statin	88 (64.2)	213 (66.4)	155 (65.1)	0.898
Non-cardiovascular medication prior to admission				
Steroid tablets	10 (7.3)	8 (2.3)	8 (3.4)	**0.043**

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).

† Kruskal-Wallis test was used for continuous variables and χ^2 test for categorical variables.

*measured at WIG and GRI

11.3.4 Association of the *CYP11B2* -344T/C polymorphism with prognosis

During the course of follow-up, 292 deaths occurred in the patients of the overall hospitalised cohort. The distribution of the events according to -344T/C genotypes in these patients is displayed in Table 11-4 below.

Table 11-4. Number and percentage (%) of events according to CY11B2 -344T/C genotypes

CYP11B2 -344 T/C genotype	TT	TC	TT
Total number of events (%)	58 (37.7%)	148 (42.9)	86 (43%)

The Kaplan-Meier survival curves for all-cause mortality were not significantly different among the three genotypes (TT versus TC versus CC) (Figure 11-3). A corresponding Cox regression analysis with the CC genotype as the reference genotype revealed no difference in the HR among patients with the TC genotype (HR, 1.141; 95% CI, 0.842 to 1.546; p=0.39) and patients with the TT genotype (HR, 1.149; 95% CI, 0.823 to 1.603; p=0.41).

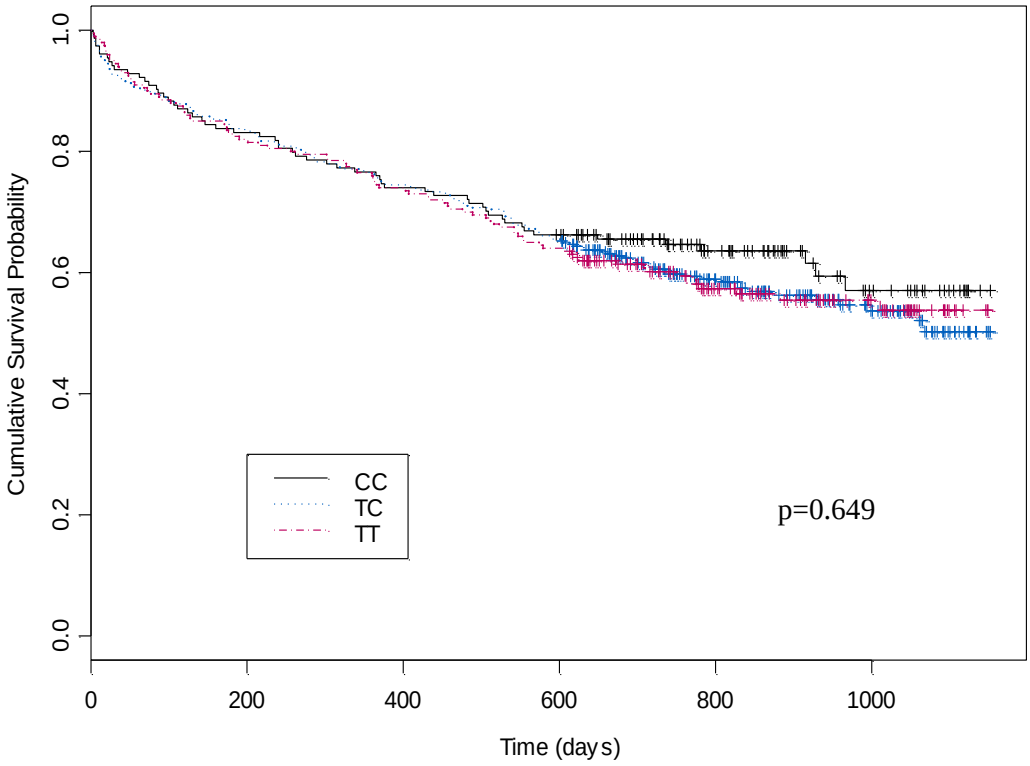


Figure 11-3. Event-free survival by *CYP11B2* -344 T/C genotypes in the overall hospitalised cohort

When patients with the C allele were pooled in one group (CC + TC) and compared with the TT patients, the Kaplan-Meier curves for all-cause mortality were not different (CC + TC versus TT) (HR, 1.047; 95% CI, 0.814 to 1.347; p=0.72).

11.3.5 Association of the *CYP11B2* IC with prognosis

For the entire cohort, the Kaplan-Meier survival curves for all-cause mortality were not significantly different among the three genotypes (Wt versus Htz versus Con) (Figure 11-4).

A corresponding Cox regression analysis with the Con genotype as the reference category revealed no difference in HR among patients with the Htz genotype (HR, 0.976; 95% CI,

0.722 to 1.319); p=0.87) and patients with the Wt genotype (HR, 0.833; 95% CI, 0.638 to 1.221; p=0.45).

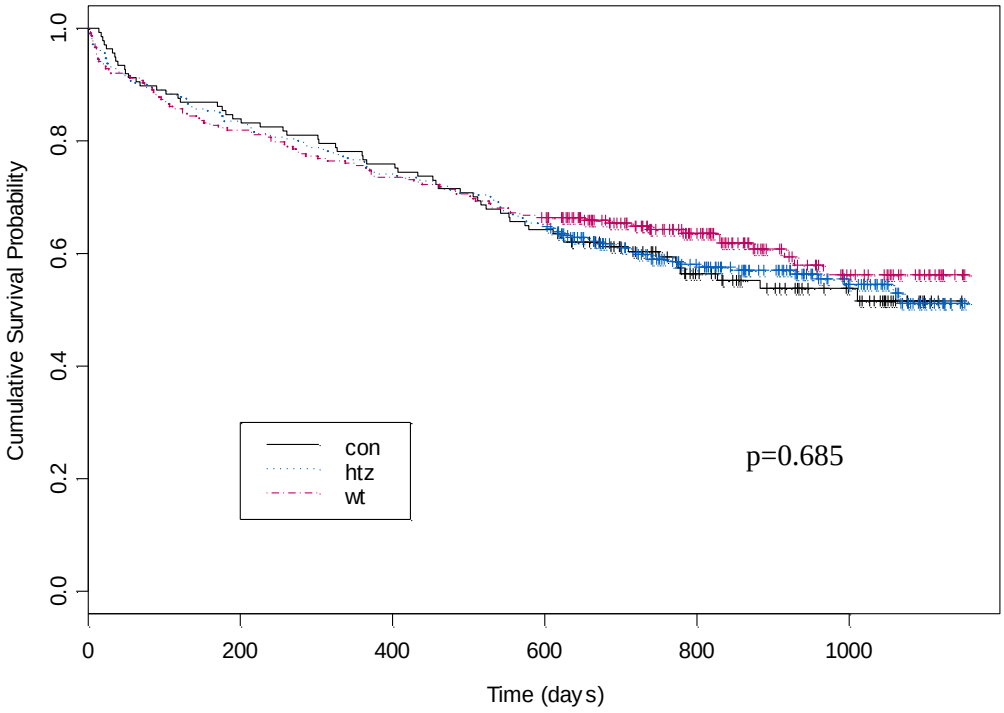


Figure 11-4. Event-free survival by *CYP11B2* IC genotypes in the overall hospitalised cohort

11.4 Discussion

11.4.1 Associations of *CYP11B2* polymorphisms with gluco- and mineralo- corticoid secretion

To the best of my knowledge, this is the first study in patients with HF to show an association between the *CYP11B2* -344T/C polymorphism and 11-deoxycortisol levels and the 11-deoxycortisol to cortisol ratio, which is an index of 11beta-hydroxylase efficiency. It has been consistently shown that the -344TT genotype is associated with increased basal and ACTH stimulated levels of plasma and urine 11-deoxycortisol and 11-deoxycortisol to cortisol ratio in healthy subjects (295) (299) (407) (408). Similarly, the TT genotype has been correlated with higher urine 11-deoxycortisol to cortisol ratio in patients with hypertension (311). The above associations have now been replicated and further extended in a population of patients with HF. Both 11-deoxycortisol levels and 11-deoxycortisol to cortisol ratio were higher in homozygotes for the T allele compared to homozygotes for the C allele, with heterozygotes being intermediate, during the hospital admission. 11-deoxycortisol is converted to cortisol by 11beta-hydroxylase in ZF. Classic 11beta-hydroxylase deficiency is characterised by a decrease in the synthesis of cortisol and a secondary increase in the secretion of 11-deoxycortisol in circulation and a higher 11-deoxycortisol to cortisol ratio. The higher 11-deoxycortisol levels and the 11-deoxycortisol to cortisol ratio seen in patients with the -344T allele are likely to reflect a relative impairment in 11beta-hydroxylase efficiency in these patients.

In contrast to 11-deoxycortisol, no differences were seen in cortisol levels among the genotype subgroups. This is in keeping with previous studies in healthy subjects and patients with hypertension, where an altered plasma level of 11-deoxycortisol but not cortisol was found among the genotypes (295) (299). Under the greater ACTH stimulation in the morning, homozygotes for the T allele appear to achieve levels of cortisol similar to heterozygotes and

homozygotes of the C allele in expense of higher 11-deoxycortisol levels. In this way, the relatively impaired efficiency of 11beta-hydroxylase is not translated into lower levels of the biologically active end product but results in higher levels of the precursor.

The association between *CYP11B2* -344 T/C polymorphism and 11beta-hydroxylase activity seen in patients with HF is in keeping with previous studies in normotensive and hypertensive individuals. *CYP11B2* and *CYP11B1* are homologous and lie in close proximity on chromosome 8 in humans. It was initially hypothesized that -344T/C polymorphism is not directly linked with the intermediate glucocorticoid phenotype and the causative locus would be probably located within *CYP11B1*; thus, a LD between the two loci would account for the above observations (299) (409). Indeed, LD has been demonstrated across the two gene regions in normal subjects and patients with hypertension resulting in three common haplotypes (312) (313). In these studies, higher 11-deoxycortisol levels were associated with the haplotypes that included the -344T allele. Two polymorphisms were further identified in the promoter coding region of *CYP11B1* in close LD with -344T/C polymorphism (314). These polymorphisms were associated with altered gene transcription in response to ACTH in vitro and 11beta-hydroxylase activity in patients with hypertension.

In contrast to the associations with glucocorticoid levels, the -344T/C polymorphism was not associated with aldosterone levels or the aldosterone to renin ratio in the current study. The -344T allele has been previously associated with higher plasma and urine levels of aldosterone (298) (300). Moreover, the same allele has been associated with a raised aldosterone to renin ratio (410). However, the genotype-phenotype associations between -344T/C polymorphism and aldosterone secretion have not been always consistent, with other studies reporting no correlation or reporting the CC genotype to be associated with increased aldosterone secretion (303) (304) (411). The reasons for the above discrepancies in the

literature are not clear and many contributing factors, as differences in the size of studies, ethnic and demographic diversity, as well as variation in the background medication, might be implicated (412). Nevertheless, in a recent meta-analysis including 2872 patients with hypertension, no correlation between the 344T/C polymorphism and aldosterone levels was shown (413).

Overall, in this study I demonstrated an association between *CYP11B2* -344 T/C polymorphism with 11beta-hydroxylase activity in patients with decompensated HF. However, no associations were seen between this polymorphism and aldosterone levels or the aldosterone to renin ratio. To my knowledge, this is the first study that reports on mineralo- and gluco-corticoid levels with respect to *CYP11B2* polymorphisms in patients with HF of Caucasian origin.

11.4.2 *CYP11B2* polymorphisms and prognosis

No association between *CYP11B2* promoter -344T/C polymorphism and mortality was found in this cohort of Caucasian patients admitted to hospital with decompensated HF. The results of my study do not confirm the previously reported associations between *CYP11B2* -344 T/C polymorphism and survival in African-American patients with advanced HF as well as in patients with MI. Apart from the *CYP11B2* -344 T/C polymorphism, the *CYP11B2* IC, which is in close LD with the -344T/C and has been previously linked with increased aldosterone secretion, was not associated also with all-cause mortality in my study.

In a genetic sub-study (GRAHF) (315) of the A-HeFT (316), the *CYP11B2* -344C allele was associated with worse event-free survival in African-Americans with severe HF. The A-HeFT was a double-blinded randomised trial of the addition of a fixed combination of isosorbide dinitrate and hydralazine or placebo on top of standard therapy in African-

Americans with advanced HF. All patients were in NYHA class III-IV with LVEF of <35% and were taking an ACE inhibitor, diuretic or digoxin. The trial was additionally stratified to include patients receiving or not a beta-blocker. The GRAHF was an A-HeFT sub-study designed to investigate potential associations of genetic polymorphisms with survival and interaction with the pharmacological treatment. The *CYP11B2* promoter polymorphism -344T/C was one of the first single nucleotide polymorphisms studied in the GRAHF study. Patients with the CC genotype reported to have worse and patients with the TT genotypes better hospitalisation-free survival among the -344T/C genotypes. In addition, the mortality rate was significantly lower in homozygotes for the T allele compared to heterozygotes and homozygotes for the C allele.

Similar results were reported in a cohort of patients following MI. The cohort in this study was an admixture of different ethnic background groups with those of European ethnicity comprising the majority of participants. Interestingly, the genotype frequencies in patients of European ethnicity were similar to the genotype distribution in the current study. The common finding with the GRAHF, although reporting on patients following MI, was that TT patients found to have better survival compared to patient with the CC and TC genotypes.

The reasons for the differences between the current and the above mentioned studies with regards to *CYP11B2* -344T/C polymorphism and prognosis can only be speculated. Firstly, differences in the ethnic background may account for the discrepancy in the results between this and the GRAHF study. HF phenotype differs in African-American and Caucasian patients, with history of hypertension being more prevalent in the former compared with the latter (414). Correspondingly, the genotype frequencies with regards to -344T/C polymorphism are different among patients of different ethnic background; the

-344T allele is more prevalent in African-Americans (315) and might exert more deleterious effects in these patients compared to Caucasians. Indeed, in patients with hypertension aldosterone blockers were more effective in lowering blood pressure compared to ARBs in African-Americans but not in Caucasians (415). Secondly, differences in the background therapy and potential pharmacogenetic interactions might contribute to the different results between my study and previous ones. In the GRAHF study homozygotes for the T allele had a better response to the combination of isosorbide dinitrate and hydralazine, in terms of survival, HF hospitalisation and change in quality of life. Hence, the better survival in patients with TT in the GRAHF study might at least partially represent the better response to the above agents. In contrast, my study population was predominantly treated with a beta-blocker, ACE inhibitor or ARB, which to my knowledge have not been shown to exert any pharmacogenetic interaction with *CYP11B2* polymorphisms. Finally, as the results of the GRAFH study were not further replicated by another study in HF patients, a false positive result cannot be excluded regarding the associations of *CYP11B2* -344 T/C polymorphism with outcomes.

None of the previous studies reporting on *CYP11B2* polymorphisms and outcomes included measurements of mineralo- and gluco-corticoids. The GRAHF study group aiming to identify a mechanistic link for the association between the -344T/C polymorphism and prognosis, explored the association between the above polymorphism and LV remodeling instead. The -344C allele was associated with worse LV remodeling at 6 months in patients randomised to placebo, whilst the above association was not present in patients of the treatment arm. In keeping with that finding, C allele has been associated with increased LV volumes in Japanese patients with dilated cardiomyopathy (416). However, similar to the associations of *CYP11B2* -344 T/C polymorphism with aldosterone secretion, the data regarding the effect of the above polymorphism on LV remodeling are conflicting; in a study of Black South

African patients with dilated cardiomyopathy, serial echocardiography showed that treatment with an ACE inhibitor, diuretic and digoxin (similar to the treatment of the placebo arm in the GRAHF study) resulted in improvement of LVEF in CC and TC patients compared to TT patients (417). Moreover, in a cohort of African-Americans on standard therapy for HF, the C allele predicted improved LV dimensions compared to the T allele (418). In my patients, LV remodeling was not examined and any comparisons with the above studies are not applicable. However, the discordant results with regards to associations of *CYP11B2* -344 T/C polymorphism with LV remodeling in the aforementioned studies, even within study groups of the same ethnic background, re-emphasise the possibility that other candidate polymorphisms within or near the *CYP11B2* and in LD with the above polymorphism exert a principal functional role. Although *CYP11B2* polymorphisms and specifically -344T/C have been extensively studied in the past, the impact of the above polymorphism on aldosterone secretion is uncertain, not only in molecular but also on clinical level (413). Nevertheless, the above polymorphism has been found to be in LD with polymorphisms in the *CYP11B1* and has additionally been associated with impaired activity of 11beta-hydroxylase. Thus, part of the associations previously attributed to *CYP11B2* polymorphisms, might be due to polymorphisms in *CYP11B1*, which lies in close proximity to *CYP11B2*. 11beta-hydroxylase, as mentioned before, mediates the final step in cortisol production from 11-deoxycortisol. Interestingly, cortisol has been associated with higher blood pressure, LV remodeling following MI and worse prognosis in HF (72) (270) (320). Future studies examining both *CYP11B2* and *CYP11B1* polymorphisms with respect to mineralo- and gluco- corticosteroid secretion will give further insight into corticosteroid secretion in patients with HF.

In summary, I have demonstrated no association between the *CYP11B2* -344 T/C polymorphism and survival in a cohort of Caucasian patients with HF.

Furthermore, I have identified no correlation between *CYP11B2* IC, previously linked with aldosterone secretion, and mortality in these patients. Overall, this study does not support the application of the above *CYP11B2* polymorphisms in clinical practice for the identification of Caucasian patients with HF at higher risk of death.

12. General Discussion

RAAS activity, mineralocorticoid secretion and prognosis in patients with HF

Although neurohumoral activation is considered as one of the fundamental pathophysiological features in patients with chronic HF, RAAS activity in patients with decompensated HF has yet to be fully characterised. What little information has been reported so far, comes from studies which included patients treated with some form of a RAAS inhibitor or diuretic (55) (339) (340), perplexing the interpretation of the findings. Indeed, as demonstrated in chapter 4, treatment with an ACE inhibitor/ARB or aldosterone blocker exerts discordant effects on the levels of RAAS mediators. Hence, RAAS activity during hospitalisation was described in detail in the subgroup of patients not taking a RAAS inhibitor in chapter 6. Surprisingly, there was no evidence of increased activation of RAAS in these patients, with PRC and aldosterone levels lying on average within the normal range. These findings are in keeping with previous reports showing that RAAS is not universally activated in untreated patients with congestive HF (57) (58) (60).These studies, however, included a small number of patients and did not always reported consistent results. On the other hand, the results of my study call into question the generally accepted concept of RAAS activation in patients with decompensated HF. Neurohumoral activation was reported in the early landmark trials of ACE inhibitors in advanced congestive HF, which although included patients on high doses of diuretics, led partially to the undisputed notion of RAAS activation in patients with congestive HF.

Why is RAAS activation not prominent in patients with worsening HF? In order to explain the previously reported variability of RAAS activation in patients with congestive HF, it has been suggested that the RAAS activity depends on the severity of haemodynamic compromise and the stage of HF (59). RAAS activity is greater in patients with decompensated HF with reduced cardiac output and low blood pressure. Indeed, patients with higher PRC had lower blood pressure, more frequently LVSD and remodeling during hospital

admission in this study. On the other hand, extracellular volume expansion and normal blood pressure are associated with normal or low RAAS activity. Patients included in the current study were normotensive or hypertensive with the vast majority having signs of fluid overload, features that are likely to account for the normal levels of renin and aldosterone. In addition, BNP levels were elevated during hospital admission; raised levels of natriuretic peptides are likely to contribute to the lower levels of RAAS mediators, as apart from the vasodilating and natriuretic effects, they suppress the activation of the SNS and RAAS (30) (124) (127). Overall, the expansion of extracellular fluid volume is likely to exert in combination with the activation of natriuretic counter-regulatory system, a prevailing inhibiting effect on RAAS activity, overriding the effects of RAAS stimulators during hospital admission in the current studies.

Clinical and prognostic markers of HF severity were also examined in relation to RAAS activity in patients during hospital admission in chapter 6. RAAS activity was greater, as previously mentioned, in patients with lower SBP, LVSD and dilatation, as well as with markers of renal dysfunction, showing that higher neurohumoral activity is a characteristic of patients with decompensated HF and cardiorenal syndrome. The management of these patients in clinical practice remains challenging. Reduction of fluid overload and intracardiac filling pressures with diuretics along with haemodynamic support with inotropes, if necessary, remains the initial therapeutic approach in the majority of these patients. However, this strategy is not necessarily translated into better outcomes in patients with decompensated HF despite the symptomatic relief of fluid congestion (419). Unfortunately, newer therapeutic agents failed to show mortality benefit in these patients (420). MR activation may contribute to decompensation and cardiorenal dysfunction during worsening HF. Indeed, aldosterone promotes sodium and water retention through MRs in the kidneys. Alternatively, cortisol under conditions of reduced inactivation by 11beta- HSD2 in patients with cardiorenal

syndrome may contribute to the fluid retention and worsening of HF through the epithelial MRs. Moreover, aldosterone and cortisol (the latter under conditions of altered redox state), promote vascular inflammation, oxidative stress, myocardial and renal interstitial fibrosis and exert in parallel pro-arrhythmic effects on myocardium by activation of non-epithelial MRs. In addition, MR expression is up-regulated in patients with HF and may be associated with augmented corticosteroid-induced effects (421). Thus, aldosterone blocker by attenuating the MR-induced epithelial and non-epithelial deleterious effects may be beneficial in patients with decompensated HF by reducing the risk of progressive pump failure and sudden cardiac death and potentially the worsening of renal function in the long term. However, the potential benefits of these agents might be compromised by the potential risk of hyperkalaemia or early deterioration of renal function especially in patients with chronic kidney disease. Appropriate patient selection and serial measurements of electrolytes might overcome these issues.

Diuretic therapy leads to reactive neurohumoral activation secondary to reduction in intravascular volume and in parallel to a reduction in natriuretic peptide levels due to decrease in cardiac filling pressures. These changes are seen early following diuretic treatment. It remains unclear, however, whether the dissociation between natriuretic peptides and RAAS seen after initiation of diuretic therapy persists in the long term.

In Chapter 7, I demonstrated that PRC and aldosterone levels were higher 4 to 6 weeks after discharge compared with hospital admission secondary to effective diuresis. In parallel, there was an improvement in natriuretic peptide levels as reflected by the decline in BNP concentration. Natriuretic peptides, as mentioned above, promote diuresis and vasodilation, suppress RAAS and SNS activity and enhance parasympathetic action; thus, the decline in their levels might have additionally contributed to greater RAAS activity in the medium to

long term. Increased RAAS activity is associated with volume expansion, peripheral vascular resistance and worse prognosis in patients with HF (55) (285) (353). These findings provide evidence that further suppression of RAAS by enhancing the natriuretic peptide system might be of therapeutic benefit in patients with chronic HF. Inhibition of natriuretic peptide degradation has been examined in combination with an ACE inhibitor as an alternative therapeutic strategy in patients with HF (378) (379). However, although this approach resulted in an improvement in haemodynamic parameters, it was not further developed because of an increase in the frequency of angioedema due to inhibition of bradykinin metabolism. Similar approach of inhibiting the breakdown of natriuretic peptides in combination with an ARB rather than an ACE inhibitor has been shown to decrease natriuretic peptide levels and was associated with improvement in NHYA clinical status and left atrial reverse remodeling in patients with HFpSF (380). The same strategy is currently being tested in patients with HFrSF and results with regards to potential benefit are awaited.

In chapter 7, I also showed that lower aldosterone to renin ratio in patients with stable HF not taking a RAAS inhibitor is associated with markers of HF severity. Moreover, the aldosterone to renin ratio was more discriminating than PRC alone in classifying patients according to markers of HF severity. Information with regards to the aldosterone to PRC ratio in patients with HF is sparse and to my knowledge this is a novel finding. Interestingly, aldosterone levels were lower in patients with higher PRC in the subgroup with lower aldosterone to renin ratio compared with patients with higher aldosterone and lower PRC in the higher aldosterone to renin ratio subgroup. Conversely, BNP was higher in the former compared with the latter group indicating that natriuretic peptides may suppress more the downstream rather than the upstream components of RAAS. Thus, the aldosterone to renin ratio in HF incorporates information about neurohumoral activation, as reflected by the higher PRC, and extracellular volume expansion with raised natriuretic peptides, as reflected

by lower aldosterone levels, and might exert prognostic significance in patients with chronic HF not treated with a RAAS inhibitor.

Finally, chapter 10 examined the prognostic significance of RAAS mediators in the overall cohort of patients with decompensated HF. The first important result was that PRC is a risk marker for all-cause mortality in patients with decompensated HF among a set of independent clinical and laboratory predictors. Renin through direct and indirect effects might affect HF progression and clinical outcomes. Moreover, background therapy prior to admission modified the PRC-related risk of all-cause mortality. However, treatment with a RAAS inhibitor reflects mainly existing cardiovascular disease and co-morbidities rather than the medication per se and that may contribute to the prognostic value of PRC. Lastly, the notion that further RAAS inhibition with renin inhibitor may exert beneficial effects in patients with worsening HF cannot be fully supported by the current findings; the hazard ratios observed for PRC, either as continuous or categorical variable, do not provide strong justification for that. Interestingly, the results of the ASTRONAUT study were recently published and showed that the addition of aliskiren on top of standard medical treatment in hospitalised patients with HFrSF had no effect on cardiovascular mortality or HF hospitalisation after 6 or 12 months (377). Nevertheless, a subgroup analysis revealed a potential benefit from aliskiren in combination with other RAAS inhibitors in non-diabetics with HFrSF and that remains to be further investigated in future studies.

The second result of interest in this chapter was that patients with higher aldosterone levels did not have worse prognosis compared to patients with lower aldosterone levels. That is in contrast to previous findings in patients with worsening HF. However, approximately half of these patients were taking an aldosterone blocker. This class of RAAS inhibitors is often targeted in patients with severe HF representing a marker of worse prognosis. Given in

addition that it increases aldosterone levels, it is likely to contribute to the prognostic significance of aldosterone seen in previous studies.

Glucocorticoid secretion and prognosis in patients with HF

Glucocorticoid levels were normal 24-48 hours after hospital admission in patients with decompensated HF as shown in chapter 4. Cortisol is a non-specific indicator of stress and someone would expect cortisol levels to be elevated in patients with worsening HF requiring in-hospital treatment. Previous studies reported raised cortisol levels in untreated patients with severe congestive HF (69) (70). However, other reports showed that cortisol levels were elevated one hour after admission and returned to normal twelve hours after initiation of treatment in patients with acute cardiogenic pulmonary oedema (343). Thus, it might be possible that cortisol levels were higher in my patients on hospital admission and gradually normalised one to two days after hospitalisation.

The differences in RAAS activity and other prognostic markers according to glucocorticoid secretion during hospital admission were examined in chapter 8. Cortisol levels were associated with markers of myocardial wall stress and necrosis as well as with NYHA function class. These associations are likely to reflect the stress response according to the severity of HF; however, direct effects of glucocorticoids on cardiovascular system might represent a mechanistic link between higher levels of cortisol and markers of HF severity. Indeed, cortisol has been shown to activate MRs under conditions of altered redox state, leading to vascular inflammation, myocardial necrosis and apoptosis. In addition glucocorticoids in patients with cardiorenal syndrome potentially activate MRs in epithelial tissues promoting fluid retention. The associations of "normal" cortisol levels with strong prognostic markers of HF in my study call into question the "normal range" of cortisol in patients with HF. In fact, should we reconsider what we mean by "normal" cortisol levels in

HF? Alternatively, these findings re-emphasise the potential benefit of MR antagonists, which block aldosterone- and cortisol-induced MR activation, in patients with decompensated HF.

Interestingly, patients with lower 11-deoxycortisol to cortisol ratio had lower blood pressure, higher RAAS activity and LV remodeling during hospital admission. A lower 11-deoxycortisol to cortisol ratio reflects a higher activity of the late step in glucocorticoid synthesis. Chronic ACTH stimulation has been shown to result in up-regulation of 11beta-hydroxylase activity with more efficient conversion of the substrate to the biologically active end product (388) (389). Thus, patients at a more advanced stage of LV remodeling with worse haemodynamic status, show apart from RAAS activation, chronic stimulation of the glucocorticoid synthetic pathway. This is a novel finding suggesting the presence of HPA axis stimulation in patients with features of severe HF. Glucocorticoids up-regulate the expression of alpha-1 adrenergic receptors and angiotensin II type I receptor in VSMCs, augmenting the effects of noradrenaline and angiotensin II (422) (423). That might be teleologically useful in order to maintain tissue perfusion in patients with features of worse HF as glucocorticoids exert synergistic effects with the sympathetic system and RAAS mediators on the vasculature.

Similar to the overall hospitalised cohort, glucocorticoid levels were on average within the normal range in the overall post-discharge cohort as shown in chapter 5. Cortisol levels were significantly higher in the small subgroup of patients with glucocorticoid measurements in the morning compared with the majority of patients who had blood samples collected in the afternoon at follow-up. That indicates that the circadian rhythm continues to operate in patients with chronic HF and is in contrast to previous studies that showed abolishment of the diurnal rhythm in untreated patients with chronic HF (70). Moreover, this chapter showed no

significant change in glucocorticoid secretion between hospital admission and follow-up in patients who had blood samples collected only in the morning at both time points.

The findings in chapter 5 elucidate additionally that the lower cortisol levels at the follow-up visit compared with hospital admission in the subgroup of patients not taking an oral glucocorticoid therapy or RAAS inhibitor both during hospital admission and follow-up, as shown in chapter 9, are principally due to the diurnal rhythm effect on glucocorticoid secretion. In this latter chapter, patients with stable HF with lower 11-deoxycortisol to cortisol ratio had lower blood pressure as well as higher RAAS activity, BNP levels and worse kidney function. These findings indicate that the HPA stimulation remained chronically higher in patients with features of worse HF. That might be important as glucocorticoids exert detrimental effects on the cardiovascular system through activation of the GRs and MRs. The deleterious effects of chronic RAAS and SNS activation were not fully recognised until introduction of RAAS inhibitors and beta-blockers was shown to improve survival and improve re-hospitalisation in patients with chronic HF. The results of the current study indicate that the same might apply for the HPA axis. Aldosterone synthase inhibitors exert a partial inhibiting effect on 11beta-hydroxylase (351). Thus, apart from suppressing aldosterone levels, they could also partially suppress cortisol secretion, alleviating thus the detrimental glucocorticoid effects on cardiovascular system. There are, however, concerns about inhibition of glucocorticoid secretion in the long term and safety and efficacy studies will give answers to these issues.

Finally, in chapter 10 I examined the prognostic value of glucocorticoids in patients with decompensated HF. The first result of interest with regards to glucocorticoids was that the 11-deoxycortisol to cortisol ratio, an index of 11beta-hydroxylase activity, was not correlated with prognosis. That is also keeping in line with the finding that *CYP11B2* polymorphisms,

which were associated with 11beta-hydroxylase activity, were not associated with all-cause mortality in patients with decompensated HF. Although greater 11beta-hydroxylase activity during worsening HF was associated with markers of HF severity that was not translated into worse outcomes. The activity of 11beta-hydroxylase activity might be associated with HF progression and hospitalisation rather than hard end points as death, but that cannot be answered by the current study. In the same fashion, higher cortisol levels in patients during hospital admission were not indicators of worse prognosis in the long term. These findings are in contrast to previous reports showing the prognostic value of cortisol in patients with chronic HF (72) (73). Thus, higher cortisol levels in patients with decompensated HF might represent an indicator of acute illness; indeed cortisol was univariately associated with all-cause mortality at 1 year after hospital admission. On the other hand, in patients with chronic HF they are likely to reflect the greater glucocorticoid secretion in the long term; hence, chronic exposure to higher cortisol levels might contribute to increased mortality in these patients.

***CYP11B2* polymorphisms, corticosteroid secretion and prognosis in patients with HF**

In chapter 11, I examined the differences in corticosteroid levels during hospital admission according to *CYP11B2* -344T/C and IC polymorphisms in patients of predominantly Caucasian origin with HF. This study demonstrated that indexes of relative 11beta-hydroxylase deficiency, such as higher plasma 11-deoxycortisol levels and 11-deoxycortisol to cortisol ratio, were higher in patients with the TT genotype compared to patients with the CC genotype. To the best of my knowledge this is a novel finding, extending previous findings from patients with hypertension to patients with HF.

The associations between *CYP11B2* -344T/C polymorphism and markers of 11beta-hydroxylase efficiency are likely to be due to variants within the *CYP11B1* gene, which lies

in close proximity with the *CYP11B2* gene. Indeed, tight LD along the *CYP11B2*/*CYP11B1* locus has been confirmed in previous studies; moreover, specific variants in the 5' promoter region of *CYP11B1* gene were found to be in linkage with -344T/C and IC polymorphisms and were associated with altered activity of 11beta-hydroxylase. It has been previously hypothesised that the impaired 11beta-hydroxylase activity due to specific alleles (T and con) of the above polymorphisms results in chronic compensatory increase in ACTH drive, in order to maintain cortisol within normal levels (298) (409). These subtle changes in ACTH drive, under the synergism of other genetic and environmental factors could potentially result in altered response of the ZG cells to other trophins with higher secretion of aldosterone and a higher aldosterone to renin ratio in the long term (409).

In this study, I demonstrated no differences in aldosterone levels or the aldosterone to renin ratio according to *CYP11B2* -344T/C or IC genotypes in patients with HF during hospital admission. It is possible that under these circumstances RAAS override any of the ACTH-stimulating effects on aldosterone secretion. Indeed, there was a significant association between aldosterone and PRC levels, indicating that RAAS exerts a dominant role in aldosterone secretion in patients with decompensated HF. In contrast aldosterone and cortisol were not strongly associated, indicating that ACTH exerts a less dominant effect on the regulation of aldosterone secretion in these patients. Moreover, greater ACTH drive, as indicated by the lower 11-deoxycortisol to cortisol ratio, was evident in patients with RAAS activation and features of HF severity. Thus, any potential effects of subtle changes in ACTH drive, potentially driven by *CYP11B2* polymorphisms, on aldosterone secretion might be overshadowed by the chronic RAAS and ACTH stimulation in patients with HF. Moreover, the impact of counter-regulatory pathways, such as the natriuretic peptides, on RAAS and aldosterone secretion becomes more prominent in states of fluid overload and systemic congestion. Natriuretic peptides inhibit angiotensin- and ACTH-induced aldosterone

secretion and thus, the interplay between stimulating and suppressing pathways related to mineralocorticoid secretion, may surpass any effects on aldosterone secretion due to subtle changes in ACTH levels. Finally, treatment with a RAAS inhibitor exerts discordant effects on the levels of RAAS mediators, resulting in dissociation between the up-stream and down-stream components of the pathway, affecting both aldosterone levels and aldosterone to renin ratio. Following the exclusion of patients receiving a RAAS inhibitor, no differences in aldosterone levels were identified among patients with different genotypes. However, it should be noted that almost all patients were treated with diuretics during hospital admission. The doses of diuretic treatment have not been documented in this study and likely to affect variably RAAS activity and aldosterone secretion. However, when the aldosterone secretion was studied in relation to PRC no differences in the aldosterone to renin ratio were identified among the *CYP11B2* -344T/C and IC genotypes.

The *CYP11B2* polymorphisms were further examined with regards to prognosis in patients hospitalised with HF in chapter 11. Both -344T/C and IC polymorphisms were not associated with all-cause mortality in these patients. That is in contrast with findings from the GRAHF study, which showed that -344 TT genotype is associated with better prognosis in patients of African-American origin with severe HF (315). It might be possible that differences in the ethnic background might account for the discordance in the results with the previous studies. The *CYP11B2* -344T allele is more common in African Americans than Caucasians and might exert more deleterious phenotypic effects in the former compared with the latter group. The -344TT genotype has been associated with aldosterone excess and higher aldosterone to renin ratio in African-Americans (301). Aldosterone excess in turn has been associated with endothelial dysfunction and this might account partially for the better response of TT patients to nitric oxide donor therapy in the A-HeFT substudy. In contrast, in the current study, patients were not randomised to any treatment and were predominantly treated with an

ACE/ARB and beta-blocker. Pharmacogenetic interactions were not examined in the current study; however, no known interactions between *CYP11B2* -344T/C polymorphism and these agents have been previously reported. Thus, the lack of impact of *CYP11B2* polymorphisms on the therapeutic benefit of HF modifying disease agents in the current study may contribute to the differences with previous findings.

Limitations

Several limitations should be noted in the discussion of the results of the current thesis. RAAS, mineralo- and glucocorticoid secretion was examined in a heterogeneous group of patients with HFrSF and HFpSF. Removing the "noise" by excluding patients on a RAAS inhibitor led to a modest sample size, especially at the follow-up visit that could potentially limit the power of the analyses. Plasma corticosteroid levels were only examined once during admission and at follow-up, which might not efficiently reflect the average corticosteroid synthesis, which is a highly dynamic process. Corticosteroids exert a diurnal pattern with higher levels in the morning and lower levels at night. Although blood samples were collected only in the morning (8am - 11am) during hospital admission and mainly in the afternoon (1pm -4pm) at the follow-up visit, that is unlikely to have fully prevented the impact of diurnal rhythm on the variation of corticosteroid levels measured in patients with HF. Indeed, in the studies with healthy volunteers a significant decline in glucocorticoid levels was not only seen between morning and afternoon or evening hours but also between earlier and later time points during the morning. On the other hand, 24-hour urine collections for measurements of corticosteroid excretion rates reflect better the adrenal steroid synthetic capacity; however, this is a laborious approach and cumbersome to organise in a "real world" study.

Furthermore, the timing of blood sampling was not totally standardised with relation to the duration of hospitalisation since admission. Blood samples were collected within 24-72 hours following admission; most of the patients had blood samples collected within 24 hours with the exception of patients admitted to hospital between Friday afternoon and Monday morning who had blood samples collected within 24 – 72 hours following hospital admission. That might have further increased the variation in glucocorticoid levels due to the different degree of improvement in clinical status and HF decompensation- induced stress among patients enrolled in the study. Moreover, the lack of standardisation of blood sampling timing with relation to the duration of hospitalisation likely contributed to a greater variation in the levels of RAAS mediators due to in-hospital treatment with diuretics and RAAS inhibitors.

No echocardiographic measurements related to LV structure were undertaken during the follow-up visit; that would allow for assessment if the associations between LV morphology parameters and markers of RAAS activity and glucocorticoid secretion identified during the hospital admission would be replicated in patients with stable HF. Moreover, ACTH was not measured and that might have provided additional information about the HPA axis activity in the current studies. Furthermore, there was no control for sodium intake in this study; however, my patients were under low-normal salt diet during their hospitalisation.

All cause-mortality has been used as the sole primary end-point in the survival studies of this thesis. All-cause mortality is easy to obtain in patients over long term periods of follow-up. Moreover, it is objective and unlikely to have been exposed to bias in the ascertainment of the events. Nevertheless, prognostic markers that are related with pathophysiological pathways in HF and all-cause mortality might be associated with cardiovascular but not necessarily with non-cardiovascular deaths. That might be of importance as non-cardiovascular deaths comprise a considerable proportion of all-cause mortality in patients with HFrSF following

numerous beneficial HF modifying disease therapies (424). In addition, a higher proportion of patients with HFpSF would be expected to die from non-cardiovascular causes (425); thus, the use of cardiovascular mortality as a secondary end point, especially as a composite of cardiovascular death or HF hospitalisation, might have increased sensitivity in the current studies. Nevertheless, the information provided by the ISD regarding outcomes was based on documentation of the death certificates. The lack of information from medical notes, as well as from next of kin, and the lack of cause-specific mortality adjudication led to the selection of all-cause mortality as the only end point in the current analyses. Finally, as morbidity data were not examined, combined morbidity/mortality outcomes were not used in the current studies.

Strengths

The study population was well-characterised during the hospital admission and at follow-up visit in these studies. Patients on RAAS inhibitors were excluded in the studies examining RAAS activity, glucocorticoid secretion and their inter-relations, preventing thus any alteration of existing relationships and potential confounding findings. Corticosteroids were analysed by LCMS, which has been increasingly recognised for its sensitivity and specificity, especially with regards to aldosterone, over radioimmunoassays. In addition, PRC was measured instead of PRA in this study. As PRC has been reported to be superior to PRA for evaluating HF severity (337), this represents another strength in these studies. Finally, patients with multiple comorbid conditions were not excluded in the current studies, representing a real world population.

Future studies

Future work relevant to the studies of this thesis would aim to explore:

- The prognostic significance of RAAS mediators and corticosteroids including cardiovascular outcomes, as cardiovascular death, HF hospitalisation or combined end points in patients with decompensated HF.
- The prognostic significance of corticosteroid levels in patients with HF measured in the evening or at night. That would potentially increase the prognostic power of glucocorticoids as the intra-individual variability in corticosteroid levels due to circadian rhythm is smaller in the evening compared with the morning.
- Associations of variants in the *CYP11B1* locus with markers of 11beta-hydroxylase activity and mineralocorticoid secretion.
- Associations of variants in the *CYP11B1* locus with prognostic markers

Conclusions

Levels of RAAS mediators in patients with decompensated HF not taking a RAAS inhibitor were on average within normal levels during hospital admission. PRC and aldosterone levels were higher 4-6 weeks after discharge compared to hospital admission in patients not taking a RAAS inhibitor at both time points due to decline in the extracellular fluid volume and natriuretic peptide levels. Glucocorticoid levels measured 24-48 hours after hospital admission were also within the normal range. However, higher cortisol levels were associated with strong prognostic markers in patients with decompensated HF. Moreover, lower systolic blood systolic pressure, indexes of LV remodeling and higher PRC and BNP levels were present in patients with greater HPA activation, as reflected by the lower 11-deoxycortisol to cortisol ratio. Most of these associations were also present in patients with stable HF at follow-up. PRC but not plasma aldosterone or cortisol, was an independent predictor of all-cause mortality in patients with decompensated HF. With regards to *CYP11B2* polymorphisms, -344T/C but not IC polymorphism was associated with markers of 11beta-hydroxylase efficiency during hospital admission. Finally, none of these polymorphisms was correlated with aldosterone levels or prognosis in patients with decompensated HF.

13. Appendix

Supplementary material

1. Tables

Table 13-1. Patient (n=722) characteristics of the overall cohort according to aldosterone quartiles during hospital admission

Variable	Q1 (n=139)	Q2 (n=137)	Q3 (n=137)	Q4 (n=138)	p-value†
Age (years)	75.5 (67-6 – 80.4)	75.0 (69.0 – 81.5)	75.2 (69.3 – 82.1)	73.0 (67.3 – 81.0)	0.2985
Female gender	53 (38.1)	60 (43.8)	67 (48.9)	70 (50.7)	0.147
NYHA class					
II	36 (25.9)	37 (27.0)	31 (22.6)	36 (26.1)	
III	81 (58.3)	78 (56.9)	84 (61.3)	81 (58.7)	0.9901
IV	22 (15.8)	22 (16.1)	22 (16.1)	21 (15.2)	
Medical history					
HF	59 (42.4)	59 (43.1)	73 (53.3)	69 (0.50)	0.1977
MI	69 (49.6)	73 (53.2)	59 (43.1)	54 (39.1)	**0.0806**
Angina	81 (58.3)	87 (63.5)	74 (54.0)	64 (46.4)	**0.03265**
Diabetes mellitus	51 (36.7)	40 (29.2)	46 (33.6)	38 (27.5)	0.345
Hypertension	93 (66.9)	88 (64.2)	88 (64.2)	91 (65.9)	0.9561
AF	79 (56.8)	64 (46.7)	79 (57.7)	81 (58.7)	0.1617
CVA/TIA	31 (22.3)	28 (20.4)	29 (21.2)	29 (21.0)	0.9851
Physiological measurements					
BMI (kg/m^2)	28.7 (25.2 – 34.2)	26.3 (23.2 – 32.3)	28.4 (24.5 – 32.4)	28.2 (23.4 – 33.4)	**0.03309**
Pulse rate (bpm)	84.0 (70.0 – 105.0)	84.0 (72.0 – 100.0)	81.0 (70.0 – 100.0)	88.0 (74.0 – 109.5)	0.399
SBP (mmHg)	135.0 (115.0 – 150.5)	138.0 (118.0 – 156.0)	126.0 (114.0 – 145.0)	130.5 (115.0 – 147.5)	0.08396
DBP (mmHg)	74.0 (62.5 – 86.0)	79.0 (64.0 – 90.0)	71.0 (62.0 – 80.0)	77.5 (63.0 – 90.0)	**0.03646**
Signs of fluid congestion					

Variable	Q1 (n=139)	Q2 (n=137)	Q3 (n=137)	Q4 (n=138)	p-value†
Elevated JVP	97 (69.8)	93 (67.9)	97 (70.8)	100 (72.5)	0.5993
Peripheral oedema	113 (81.3)	99 (72.3)	102 (74.5)	97 (70.3)	0.1691
ECG rhythm					
SR	72 (51.8)	82 (59.9)	74 (54.0)	72 (52.2)	0.5114
AF	62 (44.6)	52 (38.0)	55 (40.1)	58 (42.0)	0.7145
Echo measurements					
LVEDD (cm)	5.20 (4.80 – 5.80)	5.30 (4.70 – 5.97)	5.20 (4.60 – 6.00)	5.10 (4.70 – 5.90)	0.9241
Dilated left ventricle	33 (31.1)	44 (44.4)	36 (38.7)	39 (40.2)	0.2583
LVH	46 (43.8)	44 (44.4)	40 (44.0)	42 (43.3)	0.9989
LVSD	66 (62.9)	70 (70.7)	61 (64.9)	70 (72.2)	0.4315
Laboratory measurements (blood)					
BNP (pg/ml)	827 (330 – 1731)	836 (393 – 1731)	785 (363 – 1550)	944 (396 – 1989)	0.6239
Troponin I ≥ 0.04 (µg/L)*	69 (48.9)	61 (44.5)	67 (49.0)	67 (48.6)	0.86
Sodium (mmol/L)	138.0 (135.0 – 140.5)	138.0 (135.0 – 140.0)	139.0 (136.0 – 141.0)	137.0 (135.0 – 140.0)	0.06683
Potassium (mmol/L)	4.2 (3.9 – 4.5)	4.1 (3.9 – 4.5)	4.2 (3.9 – 4.6)	4.1 (3.7 – 4.5)	0.171
Urea (mmol/L)	8.9 (6.2 – 12.2)	8.5 (6.2 – 11.8)	9.7 (7.0 – 11.6)	8.7 (6.9 – 12.0)	0.7053
Creatinine (µmol/L)	104.0 (81.0 – 103.0)	105.0 (85.0 – 135.0)	114.0 (88.0 – 146.0)	111.5 (87.0 – 140.3)	0.2494
eGFR (ml/min/1.73m^2)	60.0 (42.0 – 60.0)	57.0 (44.0 – 60.0)	51.0 (39.0 – 60.0)	53.5 (38.0 – 60.0)	**0.04437**
eGFR <60ml/min/1.73m^2	66 (47.5)	75 (54.7)	88 (64.2)	84 (60.9)	**0.0271**
Cholesterol (total) (mmol/L)	3.5 (3.0 – 4.2)	3.9 (3.4 – 4.8)	4.0 (3.2 – 4.6)	3.6 (3.1 – 4.4)	**0.01017**
HDL (mmol/L)	1.0 (0.8 – 1.3)	1.0 (0.9 – 1.4)	1.0 (0.8 – 1.2)	1.0 (0.8 – 1.2)	0.1987

Variable	Q1 (n=139)	Q2 (n=137)	Q3 (n=137)	Q4 (n=138)	p-value†
CRP (mg/L)	16.0 (6.1 – 37.0)	13.5 (6.1 – 31.8)	13.0 (5.7 – 29.0)	12.0 (6.0 – 29.0)	0.5423
Cortisol (nmol/L)	306.6 (216.8 – 401.4)	322.1 (249.2 – 452.6)	329.3 (239.8 – 444.4)	365.6 (244.2 – 476.6)	0.0569
11-deoxycortisol (pmol/L)	468.8 (274.5 – 806.5)	487.1 (271.9 – 930.2)	524.4 (300.4, 928.5)	576.3 (291.8, 1135.3)	0.3254
11-deoxycortisol/cortisol (10^{-3})	1.58 (1.05 – 2.88)	1.64 (0.93 – 2.78)	1.65 (1.15 – 3.24)	1.74 (0.91 – 3.24)	0.9122
PRC (mIU/L)	33.5 (9.9 – 178.1)	28.2 (9.6 – 106.1)	59.0 (18.4 - 191.1)	95.7 (25.7 – 327.8)	**<0.001**
Aldosterone/PRC	0.29 (0.07 – 1.54)	1.80 (0.54 – 5.71)	1.92 (0.58 – 5.10)	3.19 (1.13 – 12.91)	**<0.001**
TSH (mIU/L)	2.0 (1.1 – 2.8)	1.8 (1.1 – 2.7)	1.8 (1.0 – 2.8)	1.6 (1.0 – 2.8)	0.6249
Haemoglobin (g/dl)	11.9 (10.6 – 13.1)	12.1 (10.7 – 13.4)	12.3 (10.7 – 13.7)	12.3 (10.6 – 13.8)	0.4247
Cardiovascular medication					
Diuretic	93 (66.9)	88 (64.2)	102 (74.5)	105 (76.1)	0.08819
ACE inhibitor	84 (60.4)	66 (48.2)	68 (49.6)	59 (42.8)	**0.0277**
ACE inhibitor or ARB	98 (70.5)	83 (60.6)	82 (59.9)	68 (49.3)	**0.004571**
Beta blocker	77 (55.4)	68 (49.6)	74 (54.0)	59 (42.8)	0.1471
Aldosterone antagonist	8 (5.8)	5 (3.6)	7 (5.1)	16 (1.2)	**0.04119**
Digoxin	23 (16.5)	20 (14.6)	27 (19.7)	25 (18.1)	0.7112
Anti-arrhythmic	2 (1.4)	4 (2.9)	10 (7.3)	8 (5.8)	0.07018
Aspirin	82 (59.0)	71 (51.8)	77 (56.2)	65 (47.1)	0.211
Statin	101 (72.7)	88 (64.2)	88 (64.2)	88 (63.8)	0.3294

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).
† Kruskal-Wallis test was used for continuous variables and χ^2 test for categorical variables.
*measured at WIG and GRI

Table 13-2. Patient (n=722) characteristics of the overall cohort according to PRC quartiles during hospital admission

Variable	Q1 (n=173)	Q2 (n=172)	Q3 (n=172)	Q4 (n=172)	p-value†
Age (years)	74.4 (69.9 – 81.5)	76.1 (67.5 – 81.5)	73.7 (68.9 – 67.5)	74.6 (67.5 – 80.7)	0.5981
Female gender	94 (54.3)	72 (41.9)	81 (47.1)	66 (38.4)	**0.01801**
NYHA class					
II	50 (28.9)	52 (30.2)	38 (22.1)	31 (18.0)	
III	100 (57.8)	98 (57.0)	107 (62.2)	104 (60.5)	**0.04925**
IV	23 (13.3)	22 (12.8)	27 (15.7)	37 (21.5)	
Medical history					
HF	48 (27.7)	65 (37.7)	75 (43.6)	114 (66.3)	**<0.001**
MI	61 (35.3)	73 (42.4)	78 (45.3)	93 (54.0)	**0.005328**
Angina	83 (48.0)	93 (54.1)	93 (54.1)	109 (63.4)	**0.03794**
Diabetes mellitus	42 (24.3)	50 (29.1)	55 (32.0)	69 (40.1)	**0.01404**
Hypertension	117 (67.6)	119 (69.2)	119 (69.2)	104 (60.4)	0.2585
AF	112 (64.7)	88 (51.2)	85 (49.4)	89 (51.7)	**0.01557**
CVA/TIA	38 (22.0)	41 (23.8)	28 (16.3)	42 (24.4)	0.2406
Physiological measurements					
BMI (kg/m^2)	27.4 (23.9 – 33.7)	27.7 (23.9 – 32.2)	28.8 (24.1 – 33.2)	28.1 (24.5 – 32.3)	0.786
Pulse rate (bpm)	88 (72 – 107)	88 (72 – 108)	88 (72 – 106)	84 (70 – 100)	0.3044
SBP (mmHg)	143.0 (125.0 – 163.0)	137.5 (122.0 – 155.0)	132.0 (116.0 – 149.3)	117.0 (104.0 – 135.0)	**<0.001**
DBP (mmHg)	83.0 (70.0 – 95.0)	75.0 (64.8 – 88.8)	74.5 (62.8 – 82.3)	67.5 (58.0 – 78.3)	**<0.001**
Signs of fluid congestion					

Variable	Q1 (n=173)	Q2 (n=172)	Q3 (n=172)	Q4 (n=172)	p-value†
Elevated JVP	115 (77.7)	123 (78.8)	120 (77.9)	128 (81.5)	0.8337
Peripheral oedema	130 (75.1)	135 (78.5)	123 (71.5)	130 (75.6)	0.5194
ECG rhythm					
SR	76 (43.9)	97 (56.4)	103 (59.9)	104 (60.5)	**0.006034**
AF	93 (53.8)	69 (40.1)	63 (36.6)	56 (32.6)	**<0.001**
Echo measurements					
LVEDD (cm)	5.13 (4.51 – 5.68)	5.04 (4.50 – 5.70)	5.10 (4.70 – 5.96)	5.40 (4.80 – 6.20)	**0.02205**
Dilated left ventricle	37 (29.1)	42 (35.0)	48 (38.7)	57 (48.3)	**0.01814**
LVH	65 (51.2)	59 (49.6)	59 (48.0)	32 (27.4)	**<0.001**
LVSD	80 (63.0)	76 (63.9)	79 (63.7)	91 (76.5)	0.07763
Laboratory measurements (blood)					
BNP (pg/ml)	974 (495 – 1934)	874 (414 – 1608)	698 (324 – 1621)	832 (338 – 1838)	0.1068
Troponin I ≥ 0.04 (µg/L)*	69 (39.9)	71 (41.3)	86 (50.0)	89 (51.7)	0.0569
Sodium (mmol/L)	139 (137 – 141)	138 (136 – 141)	138 (135 – 140)	137 (134 – 139)	**<0.001**
Potassium (mmol/L)	4.1 (3.8 – 4.5)	4.2 (3.8 – 4.5)	4.3 (3.9 – 4.6)	4.2 (3.8 – 4.5)	0.2069
Urea (mmol/L)	7.0 (5.6 – 9.6)	8.2 (6.0 – 11.7)	9.5 (7.3 – 13.3)	10.6 (7.8 – 15.6)	**<0.001**
Creatinine (µmol/L)	92.0 (77.0 – 114.0)	109.0 (85.8 – 131.3)	113.0 (88.8 – 151.0)	116.0 (92.0 – 151.3)	**<0.001**
eGFR (ml/min/1.73m^2)	60.0 (49.0 – 60.0)	56.0 (41.8 – 60.0)	55.5 (35.0 – 60.0)	52.0 (38.8 – 60.0)	**<0.001**
eGFR <60ml/min/1.73m^2	70 (40.4)	96 (55.8)	105 (61.0)	112 (65.1)	**<0.001**
Cholesterol (total) (mmol/L)	3.75 (3.00 – 4.68)	3.70 (3.15 – 4.60)	3.60 (3.10 – 4.50)	3.60 (3.10 – 4.30)	0.7315
HDL (mmol/L)	1.1 (0.9 – 1.4)	1.0 (0.8 – 1.3)	1.0 (0.8 – 1.4)	0.9 (0.8 – 1.2)	**0.03062**

Variable	Q1 (n=173)	Q2 (n=172)	Q3 (n=172)	Q4 (n=172)	p-value†
CRP (mg/L)	9.3 (4.6 – 21.5)	15.0 (6.0 – 28.0)	16.0 (7.0 – 45.5)	18.0 (8.8 – 37.0)	**<0.001**
Cortisol (nmol/L)	307.2 (218.2 – 429.6)	323.3 (213.1 – 429.7)	318.0 (246.7 – 440.9)	339.8 (255.4 – 463.7)	0.141
11-deoxycortisol (pmol/L)	502.5 (336.6 – 959.7)	511.3 (288.4 – 932.0)	461.6 (249.2 – 939.0)	470.5 (249.9 – 797.1)	0.439
11-deoxycortisol/cortisol (10^{-3})	1.83 (1.34 – 2.86)	1.79 (1.06 – 3.01)	1.44 (0.85 – 3.01)	1.41 (0.81 – 2.34)	**0.00521**2
Aldosterone (pmol/L)	56.4 (25.8 – 111.3)	71.6 (31.9 – 116.5)	84.0 (45.0 – 172.9)	90.2 (32.2 – 209.2)	**<0.001**
Aldosterone/PRC	7.63 (4.03 – 14.05)	2.62 (1.03 – 4.97)	1.18 (0.54 – 1.94)	1.29 (0.04 – 0.32)	**<0.001**
TSH (mIU/L)	0.8 (1.0 – 2.9)	1.8 (1.1 – 2.6)	1.7 (1.0 – 2.9)	1.7 (1.1 – 2.8)	0.9581
Haemoglobin (g/dl)	12.3 (11.2 – 13.8)	12.0 (10.3 – 13.4)	12.2 (10.3 – 13.5)	11.7 (10.4 – 13.5)	0.06531
Cardiovascular medication prior to admission					
Diuretic	97 (56.0)	112 (65.1)	119 (69.2)	148 (86.0)	**<0.001**
ACE inhibitor	75 (43.4)	71 (41.3)	82 (47.7)	110 (64.0)	**<0.001**
ACE inhibitor or ARB	85 (49.1)	86 (50.0)	103 (59.9)	134 (77.9)	**<0.001**
Beta blocker	95 (54.9)	87 (50.6)	74 (43.0)	76 (44.2)	0.09087
Aldosterone antagonist	6 (3.5)	3 (1.7)	13 (7.6)	25 (14.5)	**<0.001**
Digoxin	29 (16.8)	22 (12.8)	26 (15.1)	36 (20.9)	0.2184
Anti-arrhythmic	8 (4.6)	6 (3.5)	7 (4.1)	7 (4.1)	0.9627
Aspirin	90 (52.0)	91 (52.9)	99 (57.6)	89 (51.7)	0.676
Statin	104 (60.1)	114 (62.3)	111 (64.5)	122 (70.9)	0.2055

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).

† Kruskal-Wallis test was used for continuous variables and χ^2 test for categorical variables.

*measured at WIG and GRI

Table 13-3. Patient (n=722) characteristics of the overall cohort according to aldosterone to PRC quartiles during hospital admission

Variable	Q1 (n=136)	Q2 (n=135)	Q3 (n=135)	Q4 (n=136)	p-value†
Age (years)	75.1 (67.4 – 80.3)	74.6 (68.2 – 80.9)	74.4 (67.8 – 80.7)	74 .3 (69.0 – 81.8)	0.8292
Female gender	53 (39.0)	57 (42.2)	62 (45.9)	75 (44.8)	**0.04532**
NYHA class					
II	29 (21.3)	28 (20.7)	41 (30.4)	41 (30.1)	0.1933
III	82 (60.3)	81 (60.0)	75 (55.5)	80 (58.8)	
IV	25 (18.4)	26 (19.3)	19 (14.4)	15 (11.0)	
Medical history					
HF	87 (64.0)	67 (49.6)	54 (40.0)	48 (53.5)	**<0.001**
MI	76 (55.9)	72 (53.3)	58 (53.0)	43 (31.6)	**<0.001**
Angina	92 (67.6)	76 (56.3)	69 (51.1)	63 (46.3)	**0.003138**
Diabetes mellitus	54 (39.7)	47 (34.8)	40 (29.6)	32 (23.5)	**0.02868**
Hypertension	87 (64.0)	86 (63.7)	85 (63.0)	97 (71.3)	0.4303
AF	69 (50.7)	67 (49.6)	76 (56.3)	87 (64.0)	0.06924
CVA/TIA	26 (19.1)	33 (24.4)	27 (20.0)	30 (22.1)	0.7156
Physiological measurements					
BMI (kg/m^2)	28.4 (25.2 – 33.5)	28.2 (23.8 – 31.9)	27.3 (23.8 – 32.6)	27.7 (23.7 – 34.0)	0.344
Pulse rate (bpm)	82.0 (70.0 – 95.3)	84.0 (72.0 – 103.0)	88.0 (72.0 – 108.0)	87.5 (70.0 – 106.3)	0.2073
SBP (mmHg)	119.0 (106.0 – 135.5)	132.0 (115.0 – 148.5)	136.0 (120.0 – 154.5)	140.5 (124.0 – 156.0)	**<0.001**
DBP (mmHg)	69.5 (58.8 – 80.0)	132.0 (115.0 – 148.5)	136 (120 – 154.5)	140.5 (124.0 – 156.0)	**<0.001**
Signs of fluid congestion					

Variable	Q1 (n=136)	Q2 (n=135)	Q3 (n=135)	Q4 (n=136)	p-value†
Elevated JVP	90 (72.6)	101 (82.8)	97 (81.5)	91 (75.8)	0.1753
Peripheral oedema	102 (75.0)	103 (76.3)	98 (72.6)	101 (74.3)	0.9167
ECG rhythm					
SR	84 (61.8)	80 (59.3)	70 (51.9)	62 (45.6)	**0.03113**
AF	42 (30.9)	52 (38.5)	59 (43.7)	69 (50.7)	**0.007959**
Echo measurements					
LVEDD (cm)	5.3 (4.8 – 6.2)	5.0 (4.7 – 5.9)	5.3 (4.8 – 6.0)	5.1 (4.6 – 5.7)	0.1023
Dilated left ventricle	47 (47.5)	31 (33.3)	39 (43.3)	33 (33.1)	0.0522
LVH	34 (34.7)	40 (44.0)	43 (47.8)	53 (50.0)	0.1382
LVSD	68 (68.7)	68 (73.1)	57 (63.3)	69 (65.1)	0.4947
Laboratory measurements (blood)					
BNP (pg/ml)	750 (327 – 1823)	802 (379 – 1545)	957 (412 – 1825)	921 (409 – 1938)	0.3808
Troponin I ≥ 0.04 (µg/L)*	64 (47.1)	75 (55.6)	62 (45.9)	57 (41.9)	0.1474
Sodium (mmol/L)	137 (135 – 140)	137 (135 – 140)	138 (136 – 141)	139 (137 – 141)	**0.007083**
Potassium (mmol/L)	4.2 (3.9 – 4.5)	4.2 (3.9 – 4.7)	4.2 (3.9 – 4.5)	4.0 (3.8 – 4.4)	**0.02731**
Urea (mmol/L)	10.4 (7.2 – 15.0)	10.0 (7.4 – 13.3)	8.7 (6.2 – 11.2)	7.6 (5.8 – 9.8)	**<0.001**
Creatinine (µmol/L)	112.0 (89.8 – 152.3)	115.0 (92.0 – 143.5)	104.0 (82.0 – 135.5)	99.5 (79.8 – 124.0)	**0.002506**
eGFR (ml/min/1.73m^2)	53.0 (38.8 – 60.0)	53.0 (36.5 – 60.0)	58.0 (41.5 – 60.0)	60.0 (44.8 – 60.0)	**0.03795**
eGFR <60ml/min/1.73m^2	53 (60.3)	86 (63.7)	71 (52.6)	68 (50.0)	0.07772
Cholesterol (total) (mmol/L)	3.6 (3.0 – 4.2)	3.7 (3.0 – 4.4)	3.9 (3.4 – 5.0)	3.7 (3.1 – 4.7)	**0.04163**
HDL (mmol/L)	0.9 (0.8 – 1.2)	0.9 (0.8 – 1.3)	1.0 (0.9 – 1.4)	1.0 (0.9 – 1.4)	0.07757

Variable	Q1 (n=136)	Q2 (n=135)	Q3 (n=135)	Q4 (n=136)	p-value†
CRP (mg/L)	16.5 (7.0 – 36.3)	14.5 (5.7 – 41.0)	13.5 (6.0 – 53.5)	10.0 (5.5 – 24.0)	**0.03178**
Cortisol (nmol/L)	316.8 (226.7 – 435.0)	332.3 (259.4 – 456.8)	348.0 (246.3 – 454.2)	317.7 (218.4 – 440.8)	0.4963
11-deoxycortisol (pmol/L)	423.3 (253.9 – 681.2)	510.6 (241.9 – 1031.5)	537.6 (319.8 – 988.4)	681.2 (305.8 – 1188.6)	**0.04625**
11-deoxycortisol/cortisol (10^{-3})	1.37 (0.87 – 2.37)	1.69 (0.94 – 3.03)	1.82 (1.08 – 2.79)	1.83 (1.16 – 3.28)	**0.004607**
Aldosterone (pmol/L)	32.1 (14.2 – 85.0)	70.3 (32.5 – 135.2)	78.1 (37.9 – 160.1)	120.1 (63.6 – 225.3)	**<0.001**
PRC (mIU/L)	452.7 (154.4 – 161)	80.2 (45.5 – 161.0)	28.9 (14.5 – 60.5)	9.0 (5.0 – 16.2)	**<0.001**
TSH (mIU/L)	1.9 (1.1 – 2.8)	1.9 (1.1 – 2.9)	1.9 (1.1 – 2.7)	1.7 (1.0 – 2.7)	0.9057
Haemoglobin (g/dl)	11.7 (10.4 – 13.1)	12.0 (10.3 – 13.8)	12.3 (11.1 – 13.7)	12.3 (11.0 – 13.5)	0.09646
Cardiovascular medication prior to admission					
Diuretic	114 (83.8)	96 (71.1)	89 (65.9)	82 (60.3)	**<0.001**
ACE inhibitor	99 (72.8)	64 (47.4)	58 (43.0)	51 (37.5)	**<0.001**
ACE inhibitor or ARB	118 (86.8)	81 (60.0)	66 (48.9)	60 (44.1)	**<0.001**
Beta blocker	66 (48.5)	67 (49.6)	72 (53.3)	68 (50.0)	0.8742
Aldosterone antagonist	15 (11.0)	10 (7.4)	9 (6.7)	2 (1.5)	**0.01682**
Digoxin	26 (19.1)	27 (20.0)	21 (15.6)	20 (14.7)	0.5883
Anti-arrhythmic	5 (3.7)	7 (5.2)	5 (3.7)	7 (5.1)	0.8737
Aspirin	83 (61.0)	70 (51.9)	74 (54.8)	65 (47.8)	0.1649
Statin	104 (76.5)	92 (68.1)	82 (60.7)	82 (60.3)	**0.01422**

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).

† Kruskal-Wallis test was used for continuous variables and χ^2 test for categorical variables.

*measured at WIG and GRI

Table 13-4. Patient (n=722) characteristics of the overall cohort according to cortisol quartiles during hospital admission

Variable	Q1 (n=154)	Q2 (n=153)	Q3 (n=153)	Q4 (n=153)	p-value†
Age (years)	71.2 (66. – 78.5)	76.1 (71.4 – 81.6)	74.5 (68.4 – 81.3)	76.0 (69.7 – 81.2)	**0.00352**
Female gender	67 (43.5)	71 (64.4)	60 (39.2)	83 (54.2)	0.05962
NYHA class					
II	50 (32.5)	39 (25.5)	36 (23.5)	31 (20.3)	0.2422
III	83 (53.9)	94 (61.4)	92 (60.1)	93 (60.8)	
IV	21 (13.6)	20 (13.1)	25 (16.3)	29 (19.0)	
Medical history					
HF	65 (42.2)	77 (50.3)	76 (49.7)	63 (41.2)	0.2304
MI	72 (46.8)	75 (49.0)	66 (43.1)	64 (41.8)	0.5692
Angina	92 (59.7)	92 (60.1)	79 (51.6)	77 (50.3)	0.1695
Diabetes mellitus	50 (32.5)	36 (23.5)	54 (35.3)	51 (33.3)	0.1201
Hypertension	101 (65.6)	100 (65.4)	93 (60.8)	108 (70.6)	0.3529
AF	77 (50.0)	87 (56.9)	78 (51.0)	93 (60.8)	0.187
CVA/TIA	33.0 (21.4)	32.0 (20.9)	32.0 (20.9)	35.0 (22.9)	0.972
Physiological measurements					
BMI (kg/m^2)	28.5 (24.5 – 34.8)	27.4 (24.2 – 31.6)	28.2 (24.2 – 32.8)	27.4 (22.9 – 32.2)	0.1226
Pulse rate (bpm)	86.5 (72.0 – 105.0)	80.0 (68.0 – 100.0)	88.0 (70.0 – 103.0)	88.0 (74.0 – 107.0)	0.06867
SBP (mmHg)	134.0 (115.0 – 153.0)	132.0 (113.0 – 147.0)	133.0 (116.0 – 152.0)	129.0 (112.0 – 150.0)	0.4903
DBP (mmHg)	72.0 (61.3 – 84.0)	73.0 (60.0 – 85.0)	79.0 (66.0 – 90.0)	75.0(62.0 – 88.0)	**0.03291**
Signs of fluid congestion					

Variable	Q1 (n=154)	Q2 (n=153)	Q3 (n=153)	Q4 (n=153)	p-value†
Elevated JVP	103 (79.8)	108 (76.6)	102 (73.9)	119 (83.8)	0.2093
Peripheral oedema	109 (70.8)	121 (79.1)	116 (75.8)	114 (74.5)	0.4071
ECG rhythm					
SR	89 (57.8)	77 (50.3)	92 (60.1)	79 (51.6)	0.2445
AF	60 (39.0)	67 (43.8)	53 (34.6)	70 (45.8)	0.1919
Echo measurements					
LVEDD (cm)	5.3 (4.9 – 6.1)	5.2 (4.8 – 5.7)	5.4 (4.7 – 6.0)	4.9 (4.5 – 5.7)	**0.04581**
Dilated left ventricle	41 (40.6)	36 (34.6)	54 (46.2)	37 (33.6)	0.1872
LVH	47 (47.0)	42 (40.8)	56 (47.9)	46 (42.2)	0.6594
LVSD	64 (63.4)	66 (63.5)	88 (75.2)	72 (65.5)	0.1802
Laboratory measurements (blood)					
BNP (pg/ml)	642 (249 – 1220)	817 (368 – 1552)	1125 (514 – 2290)	1000 (414 – 2428)	**<0.001**
Troponin I ≥ 0.04 (µg/L)*	63 (40.9)	64 (41.8)	75 (49.0)	85 (55.6)	**0.0339**
Sodium (mmol/L)	138 (135 – 141)	139 (137 – 142)	138 (135 – 140)	137 (135 – 140)	**0.002471**
Potassium (mmol/L)	4.2 (3.9 – 4.5)	4.2 (3.8 – 4.6)	4.1 (3.8 – 4.5)	4.2 (3.8 – 4.6)	0.1975
Urea (mmol/L)	7.6 (6.0 – 10.6)	9.6 (6.6 – 12.6)	8.9 (6.9 – 11.9)	8.7 (6.7 – 12.9)	**0.01519**
Creatinine (µmol/L)	95.5 (83.0 – 128.5)	113.0 (85.0 – 146.0)	111.0 (87.0 – 132.0)	109.0 (86.0 – 141.0)	0.1702
eGFR (ml/min/1.73m^2)	60.0 (45.0 – 60.0)	51.0 (38.0 – 60.0)	55 .0 (44.0 – 60.0)	56.0 (40.0, 60.0)	**0.01252**
eGFR <60ml/min/1.73m^2	69 (44.8)	97 (63.4)	90 (58.8)	86 (56.2)	**0.008457**
Cholesterol (total) (mmol/L)	4.0 (3.3 – 4.5)	3.7 (3.1 – 4.6)	3.6 (3.0 – 4.4)	3.7 (3.0 – 4.6)	0.323
HDL (mmol/L)	1.0 (0.9 – 1.4)	1.0 (0.8 -1.4)	1.0 (0.8 – 1.2)	1.0 (0.9 – 1.2)	0.6709

Variable	Q1 (n=154)	Q2 (n=153)	Q3 (n=153)	Q4 (n=153)	p-value†
CRP (mg/L)	13.0 (5.2 – 26.0)	13.0 (6.1 – 28.0)	14.0 (6.0 – 34.2)	17.0 (8.5 – 44.0)	0.05766
11-deoxycortisol (pmol/L)	263.9 (113.4 – 408.7)	430.6 (276.6 – 690.7)	523.5 (311.8 – 899.7)	1152.4 (601.0 – 2219.2)	**<0.001**
11-deoxycortisol/cortisol (10^{-3})	1.77 (0.97 – 2.98)	1.57 (0.96 – 2.53)	1.42 (0.84 – 2.37)	1.96 (1.15 – 3.93)	**<0.001**
Aldosterone (pmol/L)	70.9 (27.1 – 157.4)	60.6 (27.7 – 112.7)	80.1 (32.9 – 159.8)	80.1 (42.8 – 183.8)	**0.02294**
PRC (mIU/L)	36.0 (12.0 – 111.6)	49.7 (11.9 – 157.7)	47.5 (14.8 – 176.3)	55.0 (15.4 – 260.3)	0.2097
Aldosterone/PRC	2.11 (0.26 – 6.85)	1.33 (0.22 – 4.49)	1.64 (0.35 – 4.32)	1.54 (0.36 – 4.90)	0.5854
TSH (mIU/L)	1.6 (1.0 – 2.4)	1.9 (1.1 – 3.0)	1.8 (1.1 – 2.8)	1.7 (1.1 – 2.9)	0.552
Haemoglobin (g/dl)	12.4 (11.0 – 13.7)	12.0 (10.4 – 13.3)	12.2 (10.8 – 13.9)	11.7 (10.4 – 12.9)	**0.04772**
Cardiovascular medication prior to admission					
Diuretic	105 (68.2)	116 (75.8)	104 (68.0)	103 (67.3)	0.3196
ACE inhibitor	83 (53.9)	74 (48.4)	74 (48.4)	80 (52.3)	0.6938
ACE inhibitor or ARB	99 (64.3)	97 (63.4)	83 (54.2)	93 (60.8)	0.2651
Beta blocker	75 (48.7)	80 (52.3)	77 (50.3)	74 (48.4)	0.8982
Aldosterone antagonist	11 (7.1)	10 (6.5)	10 (6.5)	9 (5.9)	0.9776
Digoxin	22 (14.3)	30 (19.6)	29 (19.0)	26 (17.0)	0.6091
Anti-arrhythmic	6 (3.9)	5 (3.3)	7 (4.6)	7 (4.6)	0.9269
Aspirin	97 (63.0)	85 (55.6)	72 (47.1)	74 (48.4)	**0.01853**
Statin	108 (70.1)	109 (71.2)	90 (58.8)	98 (64.1)	0.07788

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).
† Kruskal-Wallis test was used for continuous variables and χ^2 test for categorical variables.
*measured at WIG and GRI

Table 13-5. Patient (n=722) characteristics of the overall cohort according to 11-deoxycortisol quartiles during hospital admission

	Q1 (n=150)	Q2 (n=150)	Q3 (n=150)	Q4 (n=150)	p-value†
Age (years)	73.7 (66.6 – 80.9)	75.6 (67.9 – 81.4)	75.1 (68.5 – 80.9)	74.5 (69.9 – 80.2)	0.6126
Female gender	70 (46.7)	65 (43.3)	66 (44.0)	72 (48.0)	0.8301
NYHA class					
II	37 (24.7)	39 (26.0)	35 (23.3)	41 (27.3)	0.9681
III	90 (60.0)	86 (57.3)	93 (62.0)	84 (56.0)	
IV	23 (15.3)	25 (16.7)	22 (14.7)	25 (16.7)	
Medical history					
HF	64 (42.7)	81 (54.0)	83 (42.0)	49 (54.0)	0.1391
MI	64 (42.7)	70 (46.7)	68 (45.3)	67 (44.7)	0.9177
Angina	91 (60.7)	84 (56.0)	78 (52.0)	78 (52.0)	0.3775
Diabetes mellitus	49 (32.7)	44 (29.3)	44 (29.3)	52 (34.7)	0.6952
Hypertension	93 (62.0)	98 (65.3)	100 (66.7)	102 (68.0)	0.7243
AF	72 (48.0)	86 (57.3)	81 (54.0)	90 (60.0)	0.1819
CVA/TIA	31 (20.7)	36 (24.0)	25 (16.7)	39 (26.0)	0.221
Physiological measurements					
BMI (kg/m^2)	27.3 (23.8 – 32.1)	28.1 (24.5 – 32.8)	27.9 (23.7 – 32.8)	28.9 (24.6 – 34.1)	0.4362
Pulse rate (bpm)	84.0 (72.0 – 104.5)	86.0 (70.0 – 102.0)	86.0 (72.0 – 105.0)	84.0 (70.0 – 100.0)	0.911
SBP (mmHg)	127.5 (112.0 – 145.0)	135.0 (115.0 – 152.0)	135.0 (115.0 – 152.0)	133.5 (119.3 – 150.0)	0.1486
DBP (mmHg)	71.0 (62.0 – 83.8)	75.0 (61.3 – 88.0)	79.0 (63.0 – 90.0)	74.5 (65.0 – 87.8)	0.2317
Signs of fluid congestion					

	Q1 (n=150)	Q2 (n=150)	Q3 (n=150)	Q4 (n=150)	p-value†
Elevated JVP	98 (73.7)	107 (80.5)	106 (79.7)	113 (81.3)	0.4099
Peripheral oedema	10 (70.7)	119 (79.3)	111 (74.0)	117 (78.0)	0.2868
ECG rhythm					
SR	90 (60.0)	77 (51.3)	87 (58.0)	75 (50.0)	0.2232
AF	53 (35.3)	64 (42.7)	57 (38.0)	71 (47.3)	0.1572
Echo measurements					
LVEDD (cm)	5.55 (4.90 – 6.22)	5.18 (4.52 – 5.70)	5.10 (4.80 – 5.80)	5.00 (4.50 – 5.90)	**0.001847**
Dilated left ventricle	51 (50.5)	37 (34.6)	41 (38.3)	38 (34.5)	0.0604
LVH	35 (35.0)	50 (47.2)	48 (45.3)	56 (50.9)	0.122
LVSD	74 (74.0)	69 (63.9)	74 (69.2)	69 (62.7)	0.2812
Laboratory measurements (blood)					
BNP (pg/ml)	927 (369 – 2021)	841 (371 – 1499)	935 (385 – 1991)	797 (413 – 1618)	0.7196
Troponin I ≥ 0.04 (µg/L)*	67 (44.7)	71 (47.3)	75 (50.0)	65 (43.3)	0.6635
Sodium (mmol/L)	138 (135 – 141)	138 (135 – 141)	139 (135 – 141)	138 (135 – 140)	0.4042
Potassium (mmol/L)	4.2 (3.9 – 4.5)	4.2 (3.9 – 4.5)	4.1 (3.7 – 4.5)	4.2 (3.7 – 4.5)	0.2977
Urea (mmol/L)	9.4 (6.5 – 13.0)	8.8 (6.1 – 12.4)	8.8 (6.3 – 11.6)	8.5 (6.5 – 11.7)	0.7215
Creatinine (µmol/L)	110.0 (84.0 – 140.8)	110.5 (89.3 – 136.0)	109.5 (81.3 – 141.0)	104.0 (85.0 – 129.0)	0.7602
eGFR (ml/min/1.73m^2)	54.5 (39.3 – 60.0)	56.0 (41.0 – 60.0)	52.5 (42.0 – 60.0)	58.0 (44.0 – 60.0)	0.738
eGFR <60ml/min/1.73m^2	85 (56.7)	91 (60.7)	81 (54.0)	80 (53.3)	0.5674
Cholesterol (total) (mmol/L)	3.85 (3.13 – 4.60)	3.70 (3.13 – 4.60)	3.60 (2.95 – 4.25)	3.80 (3.10 – 4.70)	0.3093
HDL (mmol/L)	1.0 (0.8 – 1.2)	1.0 (0.9 – 1.4)	1.0 (0.8 – 1.2)	1.0 (0.8 – 1.4)	0.185

	Q1 (n=150)	Q2 (n=150)	Q3 (n=150)	Q4 (n=150)	p-value†
CRP (mg/L)	13.0 (6.4 – 33.0)	14.0 (6.0 – 30.0)	14.0 (6.1 – 28.0)	15.0 (6.0 – 36.0)	0.9255
Cortisol (nmol/L)	229.9 (143.5 – 322.9)	282.8 (211.1 – 363.9)	353.6 (294.8 – 360.9)	467.4 (360.9 – 600.6)	**<0.001**
11-deoxycortisol/cortisol (10^{-3})	0.66 (0.41 – 1.02)	1.35 (0.99 – 1.80)	1.77 (1.40 – 2.48)	3.80 (2.91 – 5.37)	**<0.001**
Aldosterone (pmol/L)	68.7 (20.2 – 276.6)	66.2 (27.9 – 145.8)	74.8 (30.3 – 127.6)	79.1 (43.0 – 189.3)	0.1253
PRC (mIU/L)	69.3 (20.2 – 276.6)	46.1 (11.8 – 175.6)	40.6 (12.7 – 182.0)	39.1 (11.1 – 146.4)	0.1723
Aldosterone/PRC	1.10 (0.24 – 3.83)	1.58 (0.26 – 3.88)	1.58 (0.24 – 5.97)	1.91 (0.55 – 5.97)	0.06807
TSH (mIU/L)	1.9 (0.1 – 2.9)	1.8 (1.1 – 2.6)	2.0 (1.2 – 3.0)	1.6 (0.9 – 2.5)	0.1211
Haemoglobin (g/dl)	12.0 (10.4 – 13.4)	12.3 (10.8 – 13.8)	12.0 (10.4 – 13.4)	12.1 (10.7 – 13.2)	0.4984
Cardiovascular medication prior to admission					
Diuretic	104 (69.3)	110 (73.3)	105 (70.0)	103 (68.7)	0.819
ACE inhibitor	79 (52.7)	78 (52.0)	74 (49.3)	73 (48.7)	0.8747
ACE inhibitor or ARB	96 (64.0)	88 (58.7)	93 (62.0)	87 (58.0)	0.6803
Beta blocker	73 (48.7)	81 (54.0)	72 (48.0)	76 (50.7)	0.7275
Aldosterone antagonist	12 (8.0)	8 (5.3)	13 (8.7)	7 (4.7)	0.4259
Digoxin	24 (16.0)	31 (20.7)	24 (16.0)	28 (18.7)	0.6637
Anti-arrhythmic	5 (3.3)	8 (5.3)	7 (4.7)	5 (3.3)	0.7706
Aspirin	80 (53.3)	87 (58.0)	76 (50.7)	76 (50.7)	0.5395
Statin	95 (63.3)	101 (67.3)	99 (66.0)	99 (66.0)	0.9051

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).
† Kruskal-Wallis test was used for continuous variables and χ^2 test for categorical variables.
*measured at WIG and GRI

Table 13-6. Patient (n=722) characteristics of the overall cohort according to 11-deoxycortisol to cortisol quartiles during hospital admission

	Q1 (n=150)	Q2 (n=150)	Q3 (n=150)	Q4 (n=150)	p-value†
Age (years)	74.9 (66.9 – 81.5)	75.9 (69.9 – 81.7)	73.6 (67.7 – 79.0)	73.7 (67.7 – 79.0)	0.6264
Female gender	69 (46.0)	63 (42.0)	63 (42.0)	78 (52.0)	0.2558
NYHA class					
II	33 (22.0)	30 (20.0)	45 (30.0)	44 (29.3)	0.3594
III	90 (60.0)	96 (64.0)	84 (56.0)	83 (55.3)	
IV	27 (18.0)	24 (16.0)	21 (14.0)	23 (15.3)	
Medical history					
HF	66 (44.0)	74 (49.3)	71 (47.3)	66 (44.0)	0.7401
MI	65 (43.3)	65 (43.3)	68 (45.3)	71 (47.3)	0.8809
Angina	87 (58.0)	87 (58.0)	71 (47.3)	86 (57.3)	0.1733
Diabetes mellitus	47 (31.3)	43 (28.7)	47 (31.3)	52 (34.7)	0.7389
Hypertension	94 (62.7)	97 (64.7)	98 (65.3)	104 (69.3)	0.6694
AF	75 (50.0)	90 (60.0)	81 (54.0)	83 (55.3)	0.3781
CVA/TIA	26 (17.3)	36 (24.0)	30 (20.0)	39 (26.0)	0.26
Physiological measurements					
BMI (kg/m^2)	27.2 (23.6 – 31.6)	27.8 (24.1 – 31.7)	28.2 (23.7 – 33.2)	29.6 (25.0 – 35.2)	**0.0250**5
Pulse rate (bpm)	86.0 (72.0 – 104.0)	84.5 (68.3 – 101.8)	86.0 (71.3 – 105.0)	86.5 (70.0 – 101.5)	0.8744
SBP (mmHg)	127.5 (110.3 – 143.0)	131.0 (114.0 – 149.5)	135.0 (117.3 – 155.8)	136.0 (120.3 – 150.0)	0.05376
DBP (mmHg)	70.0 (60.3 – 85.0)	78.0 (63.0 – 88.0)	77.5 (63.3 – 90.0)	74.0 (65.0 – 84.8)	0.1945
Signs of fluid congestion					

	Q1 (n=150)	Q2 (n=150)	Q3 (n=150)	Q4 (n=150)	p-value†
Elevated JVP	106 (77.9)	108 (77.7)	104 (81.3)	106 (78.5)	0.8901
Peripheral oedema	116 (77.3)	109 (72.7)	115 (76.7)	113 (75.3)	0.7925
ECG rhythm					
SR	87 (58.0)	73 (48.7)	87 (58.0)	82 (54.7)	0.3182
AF	56 (37.3)	68 (45.3)	57 (38.0)	64 (42.7)	0.436
Echo measurements					
LVEDD (cm)	5.5 (4.8 – 6.1)	5.2 (4.6 – 5.8)	5.1 (4.8 – 6.0)	5.0 (4.5 – 5.9)	0.139
Dilated left ventricle	52 (47.3)	40 (37.4)	39 (39.0)	36 (33.3)	0.1942
LVH	42 (38.2)	47 (44.8)	39 (39.0)	61 (57.0)	**0.02073**
LVSD	80 (72.7)	70 (65.4)	73 (72.3)	63 (58.9)	0.101
Laboratory measurements (blood)					
BNP (pg/ml)	1123 (441 – 2228)	942 (431 – 1992)	764 (315 – 1566)	722 (362 – 1457)	**0.009303**
Troponin I ≥ 0.04 (µg/L)*	75 (50.0)	73 (48.7)	65 (43.3)	65 (43.3)	0.527
Sodium (mmol/L)	138 (135 – 141)	138 (135 – 140)	139 (136 – 141)	138 (135 – 140)	**0.02555**
Potassium (mmol/L)	4.2 (3.9 – 4.5)	4.1 (3.8 – 4.5)	4.2 (3.8 – 4.5)	4.2 (3.8 – 4.5)	0.8219
Urea (mmol/L)	10.2 (7.5 – 13.7)	8.8 (6.3 – 11.7)	8.4 (6.0 – 10.9)	7.9 (6.1 – 11.4)	**<0.001**
Creatinine (µmol/L)	114.0 (89.3 – 149.8)	109.5 (85.0 – 136.0)	105.5 (85.0 – 136.8)	102.0 (80.0 – 129.8)	0.07453
eGFR (ml/min/1.73m^2)	53.0 (37.3 – 60.0)	57.0 (42.0 – 60.0)	56.0 (44.0 – 60.0)	60.0 (43.3 – 60.0)	**0.04349**
eGFR <60ml/min/1.73m^2	98 (65.3)	84 (56.0)	84 (56.0)	71 (47.3)	**0.01964**
Cholesterol (total) (mmol/L)	3.5 (3.0 – 4.4)	3.8 (3.1 – 4.6)	3.6 (3.3 – 4.4)	3.9 (3.1 – 4.7)	0.3726
HDL (mmol/L)	0.9 (0.8 – 1.2)	1.0 (0.9 – 1.3)	1.0 (0.8 – 1.3)	1.1 (0.9 – 1.5)	**0.01472**

	Q1 (n=150)	Q2 (n=150)	Q3 (n=150)	Q4 (n=150)	p-value†
CRP (mg/L)	18.0 (8.7 – 39.3)	13.0 (5.3 – 29.5)	12.0 (6.0 – 28.0)	14.0 (5.5 – 31.5)	**0.02094**
Cortisol (nmol/L)	322.9 (238.7 – 405.9)	335.2 (245.2 – 432.6)	305.0 (213.4 – 433.7)	366.7 (232.5 – 518.5)	0.06047
11-deoxycortisol (pmol/L)	183.7 (92.3 – 278.8)	434.2 (321.7 – 562.9)	659.1 (429.4 – 925.8)	1465.6 (940.8 – 2621.0)	**<0.001**
Aldosterone (pmol/L)	71.2 (38.2 – 169.2)	69.9 (27.6 – 125.2)	70.8 (33.8 – 135.1)	79.5 (36.7 – 194.3)	0.1899
PRC (mIU/L)	78.6 (28.3 – 305.9)	47.7 (12.3 – 288.1)	30.3 (9.2 – 136.9)	39.1 (14.1 – 123.2)	**<0.001**
Aldosterone/PRC	1.10 (0.21 – 3.30)	1.52 (0.23 – 5.16)	2.02 (0.69 – 6.09)	1.71 (0.48 – 5.62)	**0.0144**
TSH (mIU/L)	2.1 (1.1 – 3.0)	1.9 (1.1 – 2.9)	1.6 (1.0 – 2.7)	1.6 (1.0 – 2.6)	0.1933
Haemoglobin (g/dl)	11.9 (10.3 – 13.5)	12.1 (10.6 – 13.7)	12.2 (10.7 – 13.8)	12.1 (10.9 – 13.2)	0.7046
Cardiovascular medication prior to admission					
Diuretic	106 (70.7)	104 (69.3)	103 (68.7)	109 (72.7)	0.88
ACE inhibitor	77 (51.3)	79 (52.7)	77 (51.3)	71 (47.3)	0.8109
ACE inhibitor or ARB	92 (61.3)	92 (61.3)	91 (60.7)	89 (59.3)	0.9826
Beta blocker	74 (49.3)	76 (50.7)	80 (53.3)	72 (48.0)	0.8174
Aldosterone antagonist	10 (6.7)	12 (8.0)	8 (5.3)	10 (6.7)	0.8358
Digoxin	27 (18.0)	31 (20.7)	23 (15.3)	26 (17.3)	0.6846
Anti-arrhythmic	8 (5.3)	3 (2.0)	9 (6.0)	5 (3.3)	0.2841
Aspirin	75 (50.0)	87 (58.0)	69 (46.0)	88 (58.7)	0.07424
Statin	96 (64.0)	90 (60.0)	101 (67.3)	107 (71.3)	0.1999

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).
† Kruskal-Wallis test was used for continuous variables and χ^2 test for categorical variables.
*measured at WIG and GRI

Table 13-7. Patient (n=453) characteristics of the overall cohort by the median aldosterone concentration during follow-up

Variable	Aldosterone < 143 pmol/L (n=214)	aldosterone ≥ 143 pmol/L (n=214)	p-value†
Age (years)	73 (66 - 78)	71 (65 – 77)	0.328
Female gender	79 (36.9)	92 (43)	0.200
NYHA class			
I	5 (2.3)	7 (3.3)	0.558
II	140 (65.4)	139 (65)	0.919
III	67 (31.3)	67 (31.3)	1
IV	2 (0.9)	1 (0.5)	0.562
Medical history			
HF	88 (41.1)	88 (41.1)	1
MI	90 (42.1)	94 (43.9)	0.696
Angina	118 (55.1)	117 (54.7)	0.923
Diabetes mellitus	61 (28.5)	73 (34.1)	0.211
Hypertension	130 (60.8)	145 (67.8)	0.130
AF	105 (49.1)	117 (54.7)	0.246
CVA/TIA	42 (19.6)	42 (19.6)	1
Physiological measurements			
BMI (kg/m^2)	27.8 (24.1 - 32.9)	27.4 (23.6 - 32.2)	0.437
Pulse rate (bpm)	72 (64 - 82)	74 (65 - 88.3)	0.075
SBP (mmHg)	129 (115 - 144)	129 (113.8 - 145)	0.818
DBP (mmHg)	66 (58 - 74)	69 (58 - 79)	**0.036**
Signs of fluid congestion			
Elevated JVP	28 (15.4)	29 (15.7)	0.939
Peripheral oedema	71 (33.2)	71 (33.2)	1.000
ECG rhythm			
SR	133 (62.2)	125 (58.4)	0.429
AF	72 (33.6)	79 (36.9)	0.479
Echo measurements			
LVEF	40 (32 - 48)	41 (30 - 48)	0.990
Laboratory measurements (blood)			
BNP (pg/ml)	405.5 (237 - 808.8)	394 (177.8 - 816.8)	0.223
Troponin I ≥ 0.04 (µg/L)	36 (16.8)	39 (18.2)	0.703
Sodium (mmol/L)	140 (137 – 141)	139 (137 - 141)	**0.042**
Potassium (mmol/L)	4.1 (3.8 - 4.4)	4 (3.7 - 4.3)	**0.042**

Variable	Aldosterone < 143 pmol/L (n=214)	aldosterone ≥ 143 pmol/L (n=214)	p-value†
Urea (mmol/L)	9.1 (6.4 - 12.2)	8.2 (6.7 - 11.5)	0.503
Creatinine (µmol/L)	105.5 (88 - 130)	105.5 (87 - 134.5)	0.924
eGFR (ml/min/1.73m^2)	60 (43 - 60)	58 (43 - 60)	0.672
eGFR <60ml/min/1.73m^2	106 (49.5)	111 (51.9)	0.629
Cholesterol (total) (mmol/L)	3.9 (3.3 - 4.8)	4.1 (3.3 - 4.9)	0.549
HDL (mmol/L)	1.1 (0.9 - 1.3)	1 (0.8 - 1.4)	0.888
CRP (mg/L)	5 (2.6 - 12.3)	5.4 (2.6 - 12)	0.919
Cortisol (nmo/L)	195.7 (137.7 - 269.6)	241.1 (164.4 - 334.8)	**<0.001**
11-deoxycortisol (pmol/L)	457.6 (295 – 668)	484.2 (287.9 - 781.2)	0.215
11-deoxycortisol/cortisol (10^{-3})	2.54 (1.47 - 3.84)	2.11 (1.27 - 3.55)	0.071
PRC (mIU/L)	62.8 (24.3 - 284.6)	116.5 (37.7 - 368.3)	**0.021**
Aldosterone/PRC	1.01 (0.20 - 3.62)	2.28 (0.62 - 9.39)	**<0.001**
TSH (mIU/L	1.6 (0.9 - 2.3)	1.5 (0.9 - 2.4)	0.780
Haemoglobin (g/dl)	12.5 (11.1 - 13.6)	12.5 (11.4 - 13.6)	0.497
Cardiovascular medication			
Diuretic	209 (97.7)	212 (99.1)	0.253
ACE inhibitor	167 (78)	142 (66.4)	**0.007**
ACE inhibitor or ARB	181 (84.6)	160 (74.8)	**0.012**
Beta-blocker	158 (73.8)	134 (62.6)	**0.013**
Aldosterone antagonist	18 (8.4)	41 (19.2)	**0.001**
Digoxin	49 (22.9)	55 (25.7)	0.499
Anti-arrhythmic	6 (2.8)	17 (7.9)	**0.018**
Aspirin	125 (58.4)	117 (54.7)	0.435
Statin	163 (76.2)	157 (73.4)	0.504

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).

† Mann-Whitney test was used for continuous variables and χ^2 test for categorical variables.

Table 13-8. Patient (n=453) characteristics of the overall cohort by the median PRC during follow-up

Variable	PRC < 92.8 mIU/L (n=222)	PRC ≥ 92.8 mIU/L (n=223)	p-value†
Age (years)	73 (66 - 78)	71 (64 – 78)	0.109
Female gender	101 (45.5)	76 (34.1)	**0.014**
NYHA class			
I	9 (4)	3 (1.4)	0.078
II	147 (66.2)	138 (61.9)	0.341
III	64 (28.8)	80 (35.9)	0.112
IV	2 (0.9)	2 (0.9)	0.996
Medical history			
HF	75 (33.8)	108 (48.4)	**0.002**
MI	79 (35.6)	114 (51.1)	**0.001**
Angina	115 (51.8)	129 (57.9)	0.200
Diabetes mellitus	68 (30.6)	72 (32.3)	0.707
Hypertension	147 (66.2)	142 (63.7)	0.575
AF	127 (57.2)	105 (47.1)	**0.033**
CVA/TIA	46 (20.7)	42 (18.8)	0.617
Physiological measurements			
BMI (kg/m^2)	27.9 (24.1 - 33.1)	27.4 (23.5 - 31.6)	0.262
Pulse rate (bpm)	74 (64 - 87.3)	74 (65 - 85)	0.850
SBP (mmHg)	137 (122 - 151)	121 (109 - 135)	**<0.001**
DBP (mmHg)	70.5 (61 - 81)	63 (56 - 71)	**<0.001**
Pulse pressure (mmHg)	63.5 (50 - 81)	58 (42 - 71)	**<0.001**
Signs of fluid congestion			
Elevated JVP	23 (12.3)	37 (19.1)	0.070
Peripheral oedema	79 (35.6)	72 (32.3)	0.462
ECG rhythm			
SR	125 (56.3)	140 (62.8)	0.164
AF	90 (40.5)	71 (31.8)	0.056
Echo measurements			
LVEF	41 (33 - 48)	39.5 (29.8 - 47)	0.099
Laboratory measurements (blood)			
BNP (pg/ml)	419 (214.3 - 822.3)	349 (191 - 810)	0.152
Troponin I ≥ 0.04 (μg/L)	41 (18.5)	40 (17.9)	0.885
Sodium (mmol/L)	140 (138 - 142)	138 (136 - 140)	**<0.001**

Variable	PRC < 92.8 mIU/L (n=222)	PRC ≥ 92.8 mIU/L (n=223)	p-value†
Potassium (mmol/L)	4.1 (3.7 - 4.3)	4.1 (3.8 - 4.3)	0.372
Urea (mmol/L)	8 (6.3 - 10.7)	9.2 (6.7 - 12.8)	**0.006**
Creatinine (μmol/L)	101.5 (86 - 122)	110 (89 - 140)	**0.004**
eGFR (ml/min/1.73m^2)	60 (46 - 60)	55 (40 - 60)	**0.010**
eGFR <60ml/min/1.73m^2	97 (43.7)	127 (57)	**0.005**
Cholesterol (total) (mmol/L)	3.9 (3.3 - 5.1)	4.1 (3.4 - 4.8)	0.605
HDL (mmol/L)	1.1 (0.9 - 1.3)	1 (0.8 - 1.3)	0.131
CRP (mg/L)	5 (2.7 - 14)	5.5 (2.5 - 11)	0.707
Cortisol (nmo/L)	212 (138.6 - 294.2)	218.2 (154.4 - 298.4)	0.379
11-deoxycortisol (pmol/L)	460.3 (288.4 - 749.3)	468.5 (289.6 - 707.7)	0.948
11-deoxycortisol/cortisol (10^{-3})	2.30 (1.49 - 3.65)	2.36 (1.28 - 3.79)	0.487
Aldosterone (pmol/L)	120.7 (68.9 - 216.3)	168.2 (86.6 - 329.2)	**0.001**
Aldosterone/PRC	4.76 (2.15 - 13.7)	0.44 (0.12 - 1.24)	**<0.001**
TSH (mIU/L	1.5 (0.9 - 2.4)	1.6 (0.9 - 2.4)	0.689
Haemoglobin (g/dl)	12.4 (11.2 - 13.5)	12.6 (11.2 - 13.7)	0.542
Cardiovascular medication			
Diuretic	216 (97.3)	222 (99.6)	0.056
ACE inhibitor	150 (67.6)	175 (78.5)	**0.010**
ACE inhibitor or ARB	167 (75.2)	189 (84.8)	**0.012**
Beta-blocker	159 (71.6)	146 (65.5)	0.162
Aldosterone antagonist	14 (6.3)	49 (22)	**<0.001**
Digoxin	54 (24.3)	57 (25.6)	0.763
Anti-arrhythmic	11 (5)	14 (6.3)	0.544
Aspirin	115 (51.8)	135 (60.6)	0.063
Statin	158 (71.2)	171 (76.7)	0.186

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).

† Mann-Whitney test was used for continuous variables and χ^2 test for categorical variables.

Table 13-9. Patient (n=453) characteristics of the overall cohort by the median aldosterone to PRC during follow-up

Variable	aldosterone to PRC <1.51 (n=213)	aldosterone to PRC ≥1.51 (n=213)	p-value†
Age (years)	71 (63 - 77)	73 (67 - 79)	**0.021**
Female gender	69 (32.4)	101 (47.4)	**0.002**
NYHA class			
I	3 (1.4)	9 (4.2)	0.079
II	133 (62.4)	144 (67.6)	0.264
III	75 (35.2)	59 (27.7)	0.095
IV	2 (0.9)	1 (0.5)	0.562
Medical history			
HF	100 (47)	75 (35.2)	**0.014**
MI	104 (48.8)	80 (37.6)	**0.019**
Angina	125 (58.7)	110 (51.6)	0.144
Diabetes mellitus	75 (35.2)	58 (27.2)	0.075
Hypertension	134 (62.9)	139 (65.3)	0.614
AF	100 (47)	120 (56.3)	0.052
CVA/TIA	43 (20.2)	40 (18.8)	0.714
Physiological measurements			
BMI (kg/m^2)	27.5 (23.7 - 32)	27.6 (23.9 - 33)	0.687
Pulse rate (bpm)	72 (65 - 82)	75 (64 - 88)	0.124
SBP (mmHg)	122 (109 - 136)	137 (131.5 - 151)	**<0.001**
DBP (mmHg)	64 (56 - 72)	70 (60 - 80)	**<0.001**
Signs of fluid congestion			
Elevated JVP	36 (19.9)	21 (11.4)	**0.026**
Peripheral oedema	66 (31)	75 (35.2)	0.354
ECG rhythm			
SR	137 (64.3)	120 (56.3)	0.092
AF	64 (30.1)	86 (40.4)	**0.026**
Echo measurements			
LVEF	39 (30 - 46)	41 (33 - 49)	**0.014**
Laboratory measurements (blood)			
BNP (pg/ml)	392 (208.5 - 828.5)	415 (207 - 810.5)	0.655
Troponin I ≥ 0.04 (µg/L)	36 (16.9)	39 (18.3)	0.703
Sodium (mmol/L)	139 (136 – 141)	140 (138 - 141)	**<0.001**
Potassium (mmol/L)	4.1 (3.8 - 4.4)	4 (3.7 - 4.2)	**0.001**

Variable	aldosterone to PRC <1.51 (n=213)	aldosterone to PRC ≥1.51 (n=213)	p-value†
Urea (mmol/L)	9.2 (6.8 - 13.2)	8.1 (6.3 - 10.7)	**0.014**
Creatinine (µmol/L)	110 (91.5 - 137.5)	101 (84 – 127)	**0.004**
eGFR (ml/min/1.73m^2)	56 (42 - 60)	60 (44 - 60)	0.111
eGFR <60ml/min/1.73m^2	120 (56.3)	96 (45.1)	**0.020**
Cholesterol (total) (mmol/L)	4 (3.3 - 4.8)	4 (3.5 - 4.9)	0.243
HDL (mmol/L)	1 (0.8 - 1.3)	1.1 (0.9 - 1.4)	**0.007**
CRP (mg/L)	5.5 (2.5 - 11.8)	5 (2.6 - 12)	0.682
Cortisol (nmo/L)	209.2 (154.4 - 288.1)	220.2 (144.1 - 306.6)	0.780
11-deoxycortisol (pmol/L)	468.8 (309.5 - 729.6)	454.3 (257.9 - 735.2)	0.403
11-deoxycortisol/cortisol (10^{-3})	2.48 (1.38 - 3.78)	2.16 (1.38 - 3.67)	0.411
Aldosterone (pmol/L)	107.3 (50.7 - 220.9)	180.7 (101.9 - 299.7)	**<0.001**
PRC (mIU/L)	318.5 (118 - 961)	29.5 (11 – 68)	**<0.001**
TSH (mIU/L	1.6 (0.9 - 2.2)	1.5 (0.9 - 2.5)	0.986
Haemoglobin (g/dl)	12.6 (11.2 - 13.7)	12.4 (11.3 - 13.5)	0.973
Cardiovascular Medication			
Diuretic	211 (99.1)	208 (97.7)	0.253
ACE inhibitor	179 (84)	130 (61)	**<0.001**
ACE inhibitor or ARB	194 (91.1)	146 (68.5)	**<0.001**
Beta-blocker	146 (68.5)	145 (68.1)	0.917
Aldosterone antagonist	38 (17.8)	21 (9.9)	**0.017**
Digoxin	49 (23)	54 (25.4)	0.572
Anti-arrhythmic	10 (4.7)	13 (6.1)	0.520
Aspirin	129 (60.6)	113 (53.1)	0.118
Statin	165 (77.5)	153 (71.8)	0.181

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)

† Mann-Whitney test was used for continuous variables and χ^2 test for categorical variables.

Table 13-10. Patient (n=453) characteristics of the overall cohort according to the median cortisol value during follow-up

Variable	cortisol <215.8 nmol/L (n=213)	cortisol <215.8 nmol/L (n=214)	p-value†
Age (years)	71 (65 - 77)	73 (66 - 79)	0.082
Female gender	90 (42.3)	80 (37.4)	0.304
NYHA class			
I	8 (3.8)	4 (1.9)	0.238
II	141 (66.2)	137 (64)	0.637
III	63 (29.6)	71 (33.2)	0.423
IV	1 (0.5)	2 (0.9)	0.565
Medical history			
HF	92 (43.2)	83 (38.8)	0.354
MI	89 (41.8)	95 (44.4)	0.586
Angina	117 (54.9)	118 (55.1)	0.965
Diabetes mellitus	55 (25.8)	78 (36.5)	**0.018**
Hypertension	129 (60.6)	145 (67.8)	0.121
AF	112 (52.6)	109 (50.9)	0.733
CVA/TIA	46 (21.6)	38 (17.8)	0.318
Physiological measurements			
BMI (kg/m^2)	28 (24.5 - 32.8)	27 (23.2 - 32.2)	0.062
Pulse rate (bpm)	74 (63 - 84)	73 (65 - 87.3)	0.846
SBP (mmHg)	129 (116 - 145)	129 (111 - 144)	0.432
DBP (mmHg)	67 (59 - 76)	67 (57 – 76)	0.643
Signs of fluid congestion			
Elevated JVP	152 (84.4)	150 (84.6)	0.973
Peripheral oedema	152 (71.4)	133 (62.2)	**0.043**
Signs of fluid congestion			
Elevated JVP	28 (15.6)	29 (15.4)	0.973
Peripheral oedema	61 (28.6)	81 (37.9)	**0.043**
ECG rhythm			
SR	127 (59.6)	131 (61.2)	0.737
AF	77 (36.2)	73 (34.1)	0.659
Echo measurements			
LVEF	41 (41 - 48)	40 (30 - 47.8)	0.166
Laboratory measurements (blood)			
BNP (pg/ml)	334 (179 - 783)	471 (245.8 - 892.3)	**0.006**

Variable	cortisol <215.8 nmol/L (n=213)	cortisol <215.8 nmol/L (n=214)	p-value†
Troponin I ≥ 0.04 (µg/L)	33 (15.5)	42 (19.6)	0.262
Sodium (mmol/L)	139 (137 - 141)	139 (137 - 141)	0.688
Potassium (mmol/L)	4 (3.8 - 4.3)	4.1 (3.7 - 4.5)	0.092
Urea (mmol/L)	7.8 (6.0 - 10.7)	9.9 (7.2 - 12.6)	**<0.001**
Creatinine (µmol/L)	99 (84 - 119)	114 (91 - 141.3)	**<0.001**
eGFR (ml/min/1.73m^2)	60 (49 - 60)	53 (39 - 60)	**<0.001**
eGFR <60ml/min/1.73m^2	83 (39)	132 (61.7)	**<0.001**
Cholesterol (total) (mmol/L)	4.1 (3.5 - 4.8)	4 (3.1 - 4.9)	0.299
HDL (mmol/L)	1.1 (0.9 - 1.3)	1 (0.8 - 1.3)	0.273
CRP (mg/L)	4.3 (2.5 – 9.0)	7.4 (3.1 - 16.3)	**<0.001**
11-deoxycortisol (pmol/L)	384.2 (209.2 - 595.4)	605.5 (342.8 - 939.1)	**<0.001**
11-deoxycortisol/cortisol (10^{-3})	2.91 (1.85 - 4.3)	1.79 (1.2 - 3.1)	**<0.001**
Aldosterone (pmol/L)	120.5 (68.9 - 225.5)	171.1 (85.3 - 301)	**0.003**
PRC (mIU/L)	85.4 (233.3 - 365.5)	92.8 (29.4 - 276.4)	0.887
Aldosterone/PRC	1.45 (0.34 - 5.32)	1.59 (0.46 - 4.94)	0.360
TSH (mIU/L	1.4 (0.9 - 2.4)	1.6 (0.9 - 2.4)	0.171
Haemoglobin (g/dl)	12.6 (11.4 - 13.7)	12.3 (11.1 - 13.5)	0.085
Cardiovascular medication			
Diuretic	209 (98.1)	211 (98.6)	0.698
ACE inhibitor	157 (73.7)	151 (70.6)	0.468
ACE inhibitor or ARB	174 (81.7)	166 (77.6)	0.291
Beta blocker	145 (68.1)	145 (67.8)	0.944
Aldosterone antagonist	30 (14.1)	29 (13.6)	0.873
Digoxin	52 (24.4)	53 (24.8)	0.932
Anti-arrhythmic	10 (4.7)	13 (6.1)	0.528
Aspirin	119 (55.9)	122 (57)	0.812
Statin	154 (72.3)	166 (77.6)	0.209

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)

† Mann-Whitney test was used for continuous variables and χ^2 test for categorical variables.

Table 13-11. Patient (n=453) characteristics of the overall cohort by the median 11-deoxycortisol value during follow-up

Variable	11-deoxycortisol <465.7 pmol/L (n=209)	11-deoxycortisol ≥465.7 pmol/L (n=208)	p-value†
Age (years)	72(65 - 78)	71 (66 - 78)	0.952
Female gender	89 (42.6)	74 (35.6)	0.143
NYHA class			
I	9 (4.3)	3 (1.4)	0.080
II	142 (67.9)	129 (62)	0.205
III	151 (72.3)	135 (64.9)	0.106
IV	0 (0)	3 (1.4)	0.081
Medical history			
HF	80 (38.3)	91 (43.8)	0.256
MI	90 (43.1)	91 (43.8)	0.887
Angina	117 (56)	112 (53.9)	0.661
Diabetes mellitus	57 (27.3)	75 (36.1)	0.054
Hypertension	137 (65.6)	131 (63)	0.584
AF	98 (46.9)	115 (55.3)	0.086
CVA/TIA	44 (21.1)	39 (18.8)	0.556
Physiological measurements			
BMI (kg/m^2)	27.6 (23.3 - 32.2)	27.6 (24.3 - 32.9)	0.497
Pulse rate (bpm)	74 (64 - 86.5)	73 (65 - 84.8)	0.979
SBP (mmHg)	129 (113 - 143.5)	129 (114 - 144.8)	0.665
DBP (mmHg)	66 (56 - 76)	68 (59 - 76)	0.194
Signs of fluid congestion			
Elevated JVP	26 (14.7)	30 (16.7)	0.607
Peripheral oedema	67 (32.1)	72 (34.6)	0.580
ECG rhythm			
SR	134 (64.1)	121 (58.2)	0.213
AF	66 (31.6)	77 (37)	0.242
Echo measurements			
LVEF	42 (50-31)	39 (46-31)	**0.032**
Laboratory measurements (blood)			
BNP (pg/ml)	361 (203.5 - 776)	472 (206.3 - 950.8)	0.083
Troponin I ≥ 0.04 (µg/L)	31 (14.8)	41 (19.7)	0.187
Sodium (mmol/L)	139 (137 - 141)	140 (137 - 141)	0.658
Potassium (mmol/L)	4 (3.7 - 4.3)	4.1 (3.8 - 4.3)	0.188

Variable	11-deoxycortisol <465.7 pmol/L (n=209)	11-deoxycortisol ≥465.7 pmol/L (n=208)	p-value†
Urea (mmol/L)	8 (6.2 - 11.6)	9 (6.8 - 12.3)	0.190
Creatinine (µmol/L)	103 (87 - 129)	105.5 (88 - 133.5)	0.579
eGFR (ml/min/1.73m^2)	59 (43 - 60)	60 (44 - 60)	0.877
eGFR <60ml/min/1.73m^2	106 (50.7)	103 (49.5)	0.807
Cholesterol (total) (mmol/L)	4.1 (3.4 - 4.8)	3.9 (3.2 - 4.8)	0.426
HDL (mmol/L)	1.1 (0.9 - 1.3)	1 (0.8 - 1.3)	0.087
CRP (mg/L)	5.3 (2.7 - 11)	4.6 (2.4 - 13.8)	0.730
Cortisol (nmo/L)	186.3 (123.9 - 255.6)	265.5 (181.7 - 360.2)	**<0.001**
11-deoxycortisol/cortisol (10^{-3})	1.46 (0.89 - 2.44)	3.32 (2.26 - 4.9)	**<0.001**
Aldosterone (pmol/L)	129.3 (67.7 - 266.2)	147.8 (84 - 269.9)	0.229
PRC (mIU/L)	94.3 (28.9 - 302.9)	94.4 (24.8 - 400.2)	0.689
Aldosterone/PRC	1.55 (0.42 - 4.97)	1.40 (0.36 - 6.3)	0.972
TSH (mIU/L	1.5 (0.85 - 2.3)	1.6 (0.9 - 2.4)	0.294
Haemoglobin (g/dl)	12.4 (11.2 - 13.4)	12.7 (11.4 - 14.1)	**0.039**
Cardiovascular medication			
Diuretic	203 (97.1)	207 (99.5)	0.057
ACE inhibitor	144 (68.9)	161 (77.4)	**0.050**
ACE inhibitor or ARB	165 (79)	170 (81.7)	0.475
Beta-blocker	134 (64.1)	153 (73.6)	**0.037**
Aldosterone antagonist	28 (13.4)	31 (14.9)	0.659
Digoxin	43 (20.6)	57 (27.4)	0.102
Anti-arrhythmic	12 (5.7)	10 (4.8)	0.670
Aspirin	121 (57.9)	117 (56.3)	0.734
Statin	153 (73.2)	161 (77.4)	0.320

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage)

† Mann-Whitney test was used for continuous variables and χ^2 test for categorical variables.

Table 13-12. Patient (n=453) characteristics of the overall cohort by the median 11-deoxycortisol to cortisol during follow-up

Variable	11-deoxycortisol to cortisol <2.33 x10^{-3} (n=207)	11-deoxycortisol to cortisol ≥2.33 x10^{-3} (n=208)	p-value†
Age (years)	72 (66 - 79)	71 (65 – 77)	0.317
Female gender	85 (41.1)	76 (36.5)	0.344
NYHA class			
I	6 (2.9)	6 (2.9)	0.993
II	132 (63.8)	137 (65.9)	0.655
III	68 (32.9)	63 (30.3)	0.575
IV	1 (0.5)	2 (0.96)	0.565
Medical history			
HF	80 (38.7)	90 (43.3)	0.338
MI	89 (43)	92 (44.2)	0.800
Angina	115 (55.6)	114 (54.8)	0.878
Diabetes mellitus	65 (31.4)	66 (31.7)	0.942
Hypertension	137 (66.2)	129 (62)	0.377
AF	99 (47.8)	112 (53.9)	0.220
CVA/TIA	41 (19.8)	42 (20.2)	0.922
Physiological measurements			
BMI (kg/m^2)	26.8 (23.2 - 31.5)	28 (24.6 - 33.7)	**0.030**
Pulse rate (bpm)	73 (65 - 86)	73 (65 - 85.8)	0.672
SBP (mmHg)	125 (110 - 140)	131.5 (117.3 - 147)	**0.008**
DBP (mmHg)	66 (56 - 76)	68 (59 - 76)	0.165
Signs of fluid overload			
Elevated JVP	23 (12.5)	33 (19.1)	0.088
Peripheral oedema	78 (37.7)	60 (28.9)	0.056
ECG rhythm			
SR	135 (65.2)	120 (57.7)	0.115
AF	62 (30)	79 (38)	0.084
Echo measurements			
LVEF	41 (32 - 49)	40 (30 - 47)	0.228
Laboratory measurements (blood)			
BNP (pg/ml)	429 (245 - 920)	345.5 (175.5 - 790.8)	**0.050**
Troponin I ≥ 0.04 (µg/L)	37 (17.9)	35 (16.8)	0.778
Sodium (mmol/L)	139 (137 - 141)	140 (137 - 141)	0.770
Potassium (mmol/L)	4.0 (3.7 - 4.3)	4.1 (3.8 - 4.3)	0.228

Variable	11-deoxycortisol to cortisol <2.33 x10^{-3} (n=207)	11-deoxycortisol to cortisol ≥2.33 x10^{-3} (n=208)	p-value†
Urea (mmol/L)	9.3 (7.0 - 12.3)	8.1 (6.0 - 11.2)	**0.009**
Creatinine (µmol/L)	112 (86 - 123)	102 (86 - 123)	**0.005**
eGFR (ml/min/1.73m^2)	54 (40- 60)	60 (48 - 60)	**0.001**
eGFR <60ml/min/1.73m^2	122 (58.9)	85 (40.9)	**<0.001**
Cholesterol (total) (mmol/L)	3.9 (3.2 - 4.8)	4 (3.4 - 4.8)	0.446
HDL (mmol/L)	1.1 (0.85 - 1.3)	1 (0.8 - 1.3)	0.609
CRP (mg/L)	6.2 (2.8 - 14)	4.3 (2.5 - 9.1)	**0.011**
Cortisol (nmo/L)	255.6 (190.4 - 325.4)	180.1 (112.8 - 269.4)	**<0.001**
11-deoxycortisol (pmol/L)	322.1 (196.7 - 476.5)	674.3 (450 - 980)	**<0.001**
Aldosterone (pmol/L)	156.8 (71.4 - 293.3)	134.1 (79.6 - 232.2)	0.084
PRC (mIU/L)	92.8 (30.2 - 297.5)	94.4 (23.4 - 372.8)	0.933
Aldosterone/PRC	1.66 (0.46 - 4.72)	1.33 (0.34 - 6.21)	0.338
TSH (mIU/L	1.6 (0.9 - 2.6)	1.4 (0.85 - 2.2)	0.137
Haemoglobin (g/dl)	12.2 (11.1 - 13.4)	12.9 (11.5 - 14.2)	**<0.001**
Cardiovascular medication			
Diuretic	202 (97.6)	206 (99)	0.250
ACE inhibitor	146 (70.5)	157 (75.5)	0.256
ACE inhibitor or ARB	159 (76.8)	174 (83.7)	0.080
Beta-blocker	131 (63.3)	154 (74)	**0.018**
Aldosterone antagonist	30 (14.5)	29 (13.9)	0.872
Digoxin	43 (20.8)	57 (27.4)	0.114
Anti-arrhythmic	16 (7.7)	6 (2.9)	**0.028**
Aspirin	120 (58)	117 (56.3)	0.723
Statin	156 (75.4)	157 (75.5)	0.978

Continuous variables are presented as median (IQR). Categorical variables are presented as number (percentage).

† Mann-Whitney test was used for continuous variables and χ^2 test for categorical variables.

Table 13-13. Univariate and Multivariate predictors of all-cause mortality (PRC in quartiles)

Variable	Univariate HR (95% CI), P-value	Multivariate HR (95% CI), P-value
PRC (Quartiles)	**<0.0001**	0.0545
≤13.00	**0.4743 (0.3506, 0.66416), <0.0001**	**0.6433 (0.4457, 0.9284), 0.0184**
13.01 – 47.30	**0.5884 (0.4408, 0.7852), 0.0003**	**0.6591 (0.4688, 0.9627), 0.0165**
47.31 – 177.30	**0.7296 (0.5543, 0.9604), 0.0246**	0.8143 (0.5932, 1.1177), 0.2036
>177.30	1 (-)	1 (-)
Age	**1.0392 (1.0270, 1.0516), <0.0001**	**1.0328 (1.0191, 1.0468), <0.0001**
Sex: Male	0.9820 (0.7960, 1.2113), 0.8650	-
Heart rate	0.9970 (0.9927, 1.0013), 0.1669	-
SBP	**0.9877 (0.9835, 0.9919), <0.0001**	**0.9926 (0.9874, 0.9978), 0.0056**
Sodium	**0.9672 (0.9458, 0.9890), 0.0034**	-
eGFR	**0.9756 (0.9683, 0.9830), <0.0001**	-
Previous HF hospitalisation: yes	**1.6004 (1.2934, 1.9800), <0.0001**	-
History of COPD: yes	**1.3770 (1.1045, 1.7166), 0.0045**	**1.5195 (1.1887, 1.9425), 0.0008**
LSVD: Yes	1.1727 (0.8947, 1.5345), 0.2496	-
Albumin	**0.9535 (0.9321, 0.9753), <0.0001**	-
Haemoglobin	**0.8876 (0.8445, 0.9329), <0.0001**	**0.9419 (0.8888, 0.9982), 0.0434**
Urea	**1.0771 (1.0596, 1.0949), <0.0001**	**1.0400 (1.0179, 1.0626), 0.0003**
Troponin (≥0.04)	**1.7707 (1.3896, 2.2563), <0.0001**	1.3046 (0.9993, 1.7030), 0.0506
log(BNP)	**1.4308 (1.2829, 1.5957), <0.0001**	**1.3316 (1.1628, 1.5249), <0.0001**

Table 13-14. Univariate and Multivariate predictors of all-cause mortality (PRC split by Q2)

Variable	Univariate HR (95% CI), P-value	Multivariate HR (95% CI), P-value
PRC ≤47.30	**0.6196 (0.5012, 0.7569), <0.0001**	**0.7241 (0.5586, 0.9387), 0.0148**
Age	**1.0392 (1.0270, 1.0516), <0.0001**	**1.0326 (1.0188, 1.0466), <0.0001**
Sex: Male	0.9820 (0.7960, 1.2113), 0.8650	-
Heart rate	0.9970 (0.9927, 1.0013), 0.1669	-
SBP	**0.9877 (0.9835, 0.9919), <0.0001**	**0.9919 (0.9868, 0.9970), 0.0020**
Sodium	**0.9672 (0.9458, 0.9890), 0.0034**	-
eGFR	**0.9756 (0.9683, 0.9830), <0.0001**	-
Previous HF hospitalisation: yes	**1.6004 (1.2934, 1.9800), <0.0001**	-
History of COPD: yes	**1.3770 (1.1045, 1.7166), 0.0045**	**1.5493 (1.2144, 1.9771), 0.0004**
LSVD: Yes	1.1727 (0.8947, 1.5345), 0.2496	-
Albumin	**0.9535 (0.9321, 0.9753), <0.0001**	-
Haemoglobin	**0.8876 (0.8445, 0.9329), <0.0001**	**0.9429 (0.8898, 0.9991), 0.0465**
Urea	**1.0771 (1.0596, 1.0949), <0.0001**	**1.0399 (1.0178, 1.0624), 0.0004**
Troponin (≥0.04)	**1.7707 (1.3896, 2.2563), <0.0001**	**1.3114 (1.0045, 1.7119), 0.0463**
log(BNP)	**1.4308 (1.2829, 1.5957), <0.0001**	**1.3311 (1.1632, 1.5231), <0.0001**

Table 13-15. Univariate and Multivariate predictors of all-cause mortality (PRC split by Q3)

Variable	Univariate HR (95% CI), P-value	Multivariate HR (95% CI), P-value
PRC ≤177.30	**0.5931 (0.4735, 0.7427), <0.0001**	**0.7178 (0.5480, 0.9402), 0.0161**
Age	**1.0392 (1.0270, 1.0516), <0.0001**	**1.0323 (1.0187, 1.0462), <0.0001**
Sex: Male	0.9820 (0.7960, 1.2113), 0.8650	-
Heart rate	0.9970 (0.9927, 1.0013), 0.1669	-
SBP	**0.9877 (0.9835, 0.9919), <0.0001**	**0.9923 (0.9871, 0.9975), 0.0035**
Sodium	**0.9672 (0.9458, 0.9890), 0.0034**	-
eGFR	**0.9756 (0.9683, 0.9830), <0.0001**	-
Previous HF hospitalisation: yes	**1.6004 (1.2934, 1.9800), <0.0001**	-
History of COPD: yes	**1.3770 (1.1045, 1.7166), 0.0045**	**1.5016 (1.1758, 1.9176), 0.0011**
LSVD: Yes	1.1727 (0.8947, 1.5345), 0.2496	-
Albumin	**0.9535 (0.9321, 0.9753), <0.0001**	-
Haemoglobin	**0.8876 (0.8445, 0.9329), <0.0001**	**0.9405 (0.8875, 0.9966), 0.0380**
Urea	**1.0771 (1.0596, 1.0949), <0.0001**	**1.0434 (1.0218, 1.0655), <0.0001**
Troponin (≥0.04)	**1.7707 (1.3896, 2.2563), <0.0001**	**1.3433 (1.0324, 1.7478), 0.0280**
log(BNP)	**1.4308 (1.2829, 1.5957), <0.0001**	**1.3130 (1.1484, 1.5011), <0.0001**

Table 13-16. Univariate and Multivariate predictors of all-cause mortality (log-transformed PRC)

Variable	Univariate HR (95% CI), P-value	Multivariate HR (95% CI), P-value
Log(PRC)	**1.1050 (1.0649, 1.1467), <0.0001**	**1.0766 (1.0259, 1.1298), 0.0027**
Age	**1.0392 (1.0270, 1.0516), <0.0001**	**1.0331 (1.0194, 1.0471), <0.0001**
Sex: Male	0.9820 (0.7960, 1.2113), 0.8650	-
Heart rate	0.9970 (0.9927, 1.0013), 0.1669	-
SBP	**0.9877 (0.9835, 0.9919), <0.0001**	**0.9930 (0.9879, 0.9983), 0.0091**
Sodium	**0.9672 (0.9458, 0.9890), 0.0034**	-
eGFR	**0.9756 (0.9683, 0.9830), <0.0001**	-
Previous HF hospitalisation: yes	**1.6004 (1.2934, 1.9800), <0.0001**	-
History of COPD: yes	**1.3770 (1.1045, 1.7166), 0.0045**	**1.5224 (1.1929, 1.9428), 0.0007**
LSVD: Yes	1.1727 (0.8947, 1.5345), 0.2496	-
Albumin	**0.9535 (0.9321, 0.9753), <0.0001**	-
Haemoglobin	**0.8876 (0.8445, 0.9329), <0.0001**	**0.9417 (0.8886, 0.9979), 0.0424**
Urea	**1.0771 (1.0596, 1.0949), <0.0001**	**1.0388 (1.0169, 1.0613), 0.0005**
Troponin (≥0.04)	**1.7707 (1.3896, 2.2563), <0.0001**	1.3006 (0.9968, 1.6970), 0.0528
log(BNP)	**1.4308 (1.2829, 1.5957), <0.0001**	**1.3459 (1.1747, 1.5420), <0.0001**

Table 13-17. Univariate and Multivariate predictors of all-cause mortality (cortisol in quartiles)

Variable	Univariate HR (95% CI), P-value	Multivariate HR (95% CI), P-value
Cortisol (Quartiles)	0.1051	0.1221
≤226.644	**0.7303 (0.5361, 0.9950), 0.0464**	1.0299 (0.7283, 1.4534), 0.8677
226.045 – 322.644	**0.7025 (0.5138, 0.9604), 0.0269**	**0.6798 (0.9408, 0.9920), 0.0450**
322.645 – 444.360	0.8066 (0.5974, 1.0942), 0.1671	0.8929 (0.6284, 1.2687), 0.5273
>444.360	1 (-)	1 (-)
Age	**1.0431 (1.0302, 1.0562), <0.0001**	**1.0343 (1.0199, 1.0490), <0.0001**
Sex: Male	1.0334 (0.8264, 1.2923), 0.7733	-
Heart rate	0.9956 (0.9909, 1.0003), 0.0691	-
SBP	**0.9884 (0.9849, 0.9929), <0.0001**	**0.9905 (0.9851, 0.9958), 0.0005**
Sodium	**0.9736 (0.9501, 0.9976), 0.0314**	-
eGFR	**0.9782 (0.9704, 0.9860), <0.0001**	-
Previous HF hospitalisation: yes	**1.5433 (1.2312, 1.9375), 0.0002**	**1.4274 (1.1018, 1.8492), 0.0071**
History of COPD: yes	**1.5245 (1.2043, 1.9298), 0.0005**	**1.5097 (1.1621, 1.9612), 0.0020**
LSVD: Yes	1.1404 (0.8560, 1.5194), 0.3694	-
Albumin	**0.9552 (0.9325, 0.9785), 0.0002**	-
Haemoglobin	**0.8887 (0.8418, 0.9383), <0.0001**	0.9596 (0.9015, 1.0214), 0.1956
Urea	**1.0585 (1.0428, 1.0744), <0.0001**	**1.0509 (1.0275, 1.0747), <0.0001**
Troponin (≥0.04)	**1.7851 (1.3813, 2.3069), <0.0001**	**1.4483 (1.0917, 1.9214), 0.0102**
Log(BNP)	**1.3865 (1.2375, 1.5535), <0.0001**	**1.2632 (1.0952, 1.4569), 0.0013**

Table 13-18. Univariate and Multivariate predictors of all-cause mortality (cortisol split by Q2)

Variable	Univariate HR (95% CI), P-value	Multivariate HR (95% CI), P-value
Cortisol ≤322.644	**0.7971 (0.6379, 0.9961), 0.0461**	0.8825 (0.6843, 1.1381), 0.3354
Age	**1.0431 (1.0302, 1.0562), <0.0001**	**1.0333 (1.0188, 1.0479), <0.0001**
Sex: Male	1.0334 (0.8264, 1.2923), 0.7733	-
Heart rate	0.9956 (0.9909, 1.0003), 0.0691	-
SBP	**0.9884 (0.9849, 0.9929), <0.0001**	**0.9903 (0.9849, 0.9957), 0.0004**
Sodium	**0.9736 (0.9501, 0.9976), 0.0314**	-
eGFR	**0.9782 (0.9704, 0.9860), <0.0001**	-
Previous HF hospitalisation: yes	**1.5433 (1.2312, 1.9375), 0.0002**	**1.3926 (1.0765, 1.8016), 0.0117**
History of COPD: yes	**1.5245 (1.2043, 1.9298), 0.0005**	**1.5730 (1.2147, 2.0371), 0.0006**
LSVD: Yes	1.1404 (0.8560, 1.5194), 0.3694	-
Albumin	**0.9552 (0.9325, 0.9785), 0.0002**	-
Haemoglobin	**0.8887 (0.8418, 0.9383), <0.0001**	0.9605 (0.9031, 1.0217), 0.2011
Urea	**1.0585 (1.0428, 1.0744), <0.0001**	**1.0510 (1.0274, 1.0751), <0.0001**
Troponin (≥0.04)	**1.7851 (1.3813, 2.3069), <0.0001**	**1.4674 (1.1095, 1.9409), 0.0072**
log(BNP)	**1.3865 (1.2375, 1.5535), <0.0001**	**1.2481 (1.0843, 1.4367), 0.0020**

Table 13-19. Univariate and Multivariate predictors of all-cause mortality (cortisol split by Q3)

Variable	Univariate HR (95% CI), P-value	Multivariate HR (95% CI), P-value
Cortisol ≤444.360	**0.7456 (0.5825, 0.9543), 0.0197**	0.8554 (0.6474, 1.1302), 0.2718
Age	**1.0431 (1.0302, 1.0562), <0.0001**	**1.0328 (1.0184, 1.0475), <0.0001**
Sex: Male	1.0334 (0.8264, 1.2923), 0.7733	-
Heart rate	0.9956 (0.9909, 1.0003), 0.0691	-
SBP	**0.9884 (0.9849, 0.9929), <0.0001**	**0.9905 (0.9851, 0.9958), 0.0005**
Sodium	**0.9736 (0.9501, 0.9976), 0.0314**	-
eGFR	**0.9782 (0.9704, 0.9860), <0.0001**	-
Previous HF hospitalisation: yes	**1.5433 (1.2312, 1.9375), 0.0002**	**1.3985 (1.0806, 1.8099), 0.0108**
History of COPD: yes	**1.5245 (1.2043, 1.9298), 0.0005**	**1.5593 (1.2039, 2.0198), 0.0008**
LSVD: Yes	1.1404 (0.8560, 1.5194), 0.3694	-
Albumin	**0.9552 (0.9325, 0.9785), 0.0002**	-
Haemoglobin	**0.8887 (0.8418, 0.9383), <0.0001**	0.9615 (0.9039, 1.0228), 0.2131
Urea	**1.0585 (1.0428, 1.0744), <0.0001**	**1.0502 (1.0267, 1.0743), <0.0001**
Troponin (≥0.04)	**1.7851 (1.3813, 2.3069), <0.0001**	**1.4633 (1.1059, 1.9360), 0.0077**
log(BNP)	**1.3865 (1.2375, 1.5535), <0.0001**	**1.2556 (1.0914, 1.4445), 0.0015**

Table 13-20. Univariate and Multivariate predictors of all-cause mortality (cortisol log-transformed)

Variable	Univariate HR (95% CI), P-value	Multivariate HR (95% CI), P-value
Log(Cortisol)	1.0657 (0.8955, 1.2682), 0.4738	0.9251 (0.7643, 1.1198), 0.4245
Age	**1.0431 (1.0302, 1.0562), <0.0001**	**1.0343 (1.0199, 1.0489), <0.0001**
Sex: Male	1.0334 (0.8264, 1.2923), 0.7733	-
Heart rate	0.9956 (0.9909, 1.0003), 0.0691	-
SBP	**0.9884 (0.9849, 0.9929), <0.0001**	**0.9903 (0.9849, 0.9958), 0.0005**
Sodium	**0.9736 (0.9501, 0.9976), 0.0314**	-
eGFR	**0.9782 (0.9704, 0.9860), <0.0001**	-
Previous HF hospitalisation: yes	**1.5433 (1.2312, 1.9375), 0.0002**	**1.3823 (1.0688, 1.7876), 0.0136**
History of COPD: yes	**1.5245 (1.2043, 1.9298), 0.0005**	**1.5536 (1.1984, 2.0142), 0.0009**
LSVD: Yes	1.1404 (0.8560, 1.5194), 0.3694	-
Albumin	**0.9552 (0.9325, 0.9785), 0.0002**	-
Haemoglobin	**0.8887 (0.8418, 0.9383), <0.0001**	0.9571 (0.8994, 1.0184), 0.1663
Urea	**1.0585 (1.0428, 1.0744), <0.0001**	**1.0506 (1.0273, 1.0745), <0.0001**
Troponin (≥0.04)	**1.7851 (1.3813, 2.3069), <0.0001**	**1.5068 (1.1419, 1.9884), 0.0038**
log(BNP)	**1.3865 (1.2375, 1.5535), <0.0001**	**1.2620 (1.0963, 1.4527), 0.0012**

References

1. McMurray JJ, Adamopoulos S, Anker SD, Auricchio A, Böhm M, Dickstein K, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012. European Heart Journal. 2012; 33(14): 1787-1847.
2. Aurigemma GP, Gaasch WH. Diastolic heart failure. New England Journal of Medicine. 2004; 351(11): 1097-1105.
3. McMurray J, Stewart S. The burden of heart failure. European Heart Journal. 2002; 4(suppl D): D50-D58.
4. Bleumink GS, Knetsch AM, Sturkenboom MC, Straus SM, Hofman A, Deckers JW, et al. Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure The Rotterdam Study. European Heart Journal. 2004; 25(18): 1614-1619.
5. Ho KK, Pinsky JL, Kannel WB, Levy D. The epidemiology of heart failure: The Framingham Study. Journal of the American College of Cardiology. 1993; 22(Suppl 1): A6-A13.
6. Jessup M, Brozena S. Heart Failure. New England Journal of Medicine. 2003; 348(20): 2007-2018.
7. McCullough PA, Philbin EF, Spertus JA, Kaatz S, Sandberg KR, Weaver WD. Confirmation of a heart failure epidemic: findings from the Resource Utilization Among Congestive Heart Failure (REACH) study. Journal of the American College of Cardiology. 2002; 39(1): 60-69.
8. Cowie M, Wood D, Coats A, Thompson S, Poole-Wilson P, Suresh V, et al. Incidence and aetiology of heart failure; a population-based study. European Heart Journal. 1999; 20(6): 421-428.
9. Bursi F, Weston SA, Redfield MM, Jacobsen SJ, Pakhomov S, Nkomo VT, et al. Systolic and diastolic heart failure in the community. Journal of the American Medical Association. 2006; 296(18): 2209-2216.
10. Hogg K, Swedberg K, McMurray J. Heart failure with preserved left ventricular systolic function: epidemiology, clinical characteristics, and prognosis. Journal of the American College of Cardiology. 2004; 43(3): 317-327.
11. Yancy CW, Lopatin M, Stevenson LW, De Marco T, Fonarow GC. Clinical presentation, management, and in-hospital outcomes of patients admitted with acute decompensated heart failure with preserved systolic function: a report from the Acute Decompensated Heart Failure National Registry (ADHERE) Database. Journal of the American College of Cardiology. 2006; 47(1): 76-84.
12. Stewart S, MacIntyre K, Hole DJ, Capewell S, McMurray JJ. More 'malignant'than cancer? Five-year survival following a first admission for heart failure. European Journal of Heart Failure. 2001; 3(3): 315-322.
13. Jhund PS, MacIntyre K, Simpson CR, Lewsey JD, Stewart S, Redpath A, et al. Long-term trends in first hospitalization for heart failure and subsequent survival between 1986 and 2003. Circulation. 2009; 119(4): 515-523.
14. Lee DS, Gona P, Vasan RS, Larson MG, Benjamin EJ, Wang TJ, et al. Relation of disease pathogenesis and risk factors to heart failure with preserved or reduced ejection fraction. Circulation. 2009; 119(24): 3070-3077.
15. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. New England

Journal of Medicine. 2006; 355(3): 251-259.
16. Somaratne JB, Berry C, McMurray JJ, Poppe KK, Doughty RN, Whalley GA. The prognostic significance of heart failure with preserved left ventricular ejection fraction: a literature-based meta-analysis. European Journal of Heart Failure. 2009; 11(9): 855-862.
17. Mann DL. Mechanisms and models in heart failure: a combinatorial approach. Circulation. 1999; 100(9): 999-1008.
18. Cohn JN. Structural basis for heart failure: ventricular remodeling and its pharmacological inhibition. Circulation. 1995; 91(10): 2504-2507.
19. Olivetti G, Abbi R, Quaini F, Kajstura J, Cheng W, Nitahara JA, et al. Apoptosis in the failing human heart. New England Journal of Medicine. 1997; 336(16): 1131-1141.
20. McMurray JJ, Pfeffer MA. Heart failure. Lancet. 2005; 365(9474): 1877-1889.
21. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: part I. Circulation. 2002; 105(11): 1387-1393.
22. Burkhoff D, Maurer MS, Packer M. Heart Failure with a normal ejection fraction is it really a disorder of diastolic function? Circulation. 2003; 107(5): 656-658.
23. Eisenhofer G, Friberg P, Rundqvist B, Quyyumi AA, Lambert G, Kaye DM, et al. Cardiac sympathetic nerve function in congestive heart failure. Circulation. 1996; 93(9): 1667-1676.
24. Levine TB, Francis GS, Goldsmith SR, Simon AB, Cohn JN. Activity of the sympathetic nervous system and renin-angiotensin system assessed by plasma hormone levels and their relation to hemodynamic abnormalities in congestive heart failure. American Journal of Cardiology. 1982; 49(7): 1659-1666.
25. Francis GS. Neurohumoral mechanisms involved in congestive heart failure. American Journal of Cardiology. 1985; 55(2): A15-A21.
26. Bayliss J, Norell MS, Canepa-Anson R, Reid C, Poole-Wilson P, Sutton G. Clinical importance of the renin-angiotensin system in chronic heart failure: double blind comparison of captopril and prazosin. British Medical Journal. 1985; 290(6485): 1861-1865.
27. McMurray J, Abdullah I, Dargie HJ, Shapiro D. Increased concentrations of tumour necrosis factor in" cachectic" patients with severe chronic heart failure. British Heart Journal. 1991; 66(5): 356-358.
28. Deswal A, Petersen NJ, Feldman AM, Young JB, White BG, Mann DL. Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesnarinone trial (VEST). Circulation. 2001; 103(16): 2055-2059.
29. Burnett JC Jr, Kao PC, Hu DC, Heser DW, Heublein D, Granger J, et al. Atrial natriuretic peptide elevation in congestive heart failure in the human. Science. 1986; 231(4742): 1145-1147.
30. Raine A, Erne P, Bürgisser E, Müller FB, Bolli P, Burkart F, et al. Atrial natriuretic peptide and atrial pressure in patients with congestive heart failure. New England Journal of Medicine. 1986; 315(9): 533-537.
31. Damkjær M, Isaksson GL, Stubbe J, Jensen BL, Assersen K, Bie P. Renal renin secretion as regulator of body fluid homeostasis. Pflügers Archiv - European Journal of Physiology. 2013;465 (1): 153 -165.
32. Lapointe JY, Bell PD, Cardinal J. Direct evidence for apical Na+: 2Cl-: K+ cotransport in macula densa cells. American Journal of Physiology-Renal Physiology. 1990; 258(5): F1466-F1469.
33. DiBona GF. Neural control of the kidney: functionally specific renal sympathetic nerve fibers. American Journal of Physiology-Regulatory, Integrative and Comparative

Physiology. 2000; 279(5): R1517-R1524.
34. Brown MJ. Renin: friend or foe? Heart. 2007; 93(9): 1026-1033.
35. Ichikawa I, Harris RC. Angiotensin actions in the kidney: renewed insight into the old hormone. Kidney International. 1991; 40(4): 583-596.
36. Clemson B, Gaul L, Gubin S, Campsey D, McConville J, Nussberger J, et al. Prejunctional angiotensin II receptors. Facilitation of norepinephrine release in the human forearm. Journal of Clinical Investigation. 1994; 93(2): 684–691.
37. Scholz H, Kurtz A. Role of protein kinase C in renal vasoconstriction caused by angiotensin II. American Journal of Physiology-Cell Physiology. 1990; 259(3): C421-C426.
38. Lonn EM, Yusuf S, Jha P, Montague TJ, Teo KK, Benedict CR, et al. Emerging role of angiotensin-converting enzyme inhibitors in cardiac and vascular protection. Circulation. 1994; 90(4): 2056-2069.
39. Schieffer B, Luchtefeld M, Braun S, Hilfiker A, Hilfiker-Kleiner D, Drexler H. Role of NAD(P)H oxidase in angiotensin II-induced JAK/STAT signaling and cytokine induction. Circulation Research. 2000; 87(12):1195-1201.
40. Berry C, Hamilton CA, Brosnan MJ, Magill FG, Berg GA, McMurray JJ, et al. Investigation into the sources of superoxide in human blood vessels: angiotensin II increases superoxide production in human internal mammary arteries. Circulation. 2000; 101(18): 2206-2212.
41. Tea BS, Der Sarkissian S, Touyz RM, Hamet , deBlois D. Proapoptotic and growth-inhibitory role of angiotensin II type 2 receptor in vascular smooth muscle cells of spontaneously hypertensive rats in vivo. Hypertension. 2000; 35(5): 1069-1073.
42. Horiuchi M, Hayashida W, Akishita M, Tamura K, Daviet L, Lehtonen JYA, et al. Stimulation of different subtypes of angiotensin II receptors, AT1 and AT2 receptors, regulates STAT activation by negative crosstalk. Circulation Research. 1999; 84(8): 876-882.
43. Zisman LS, Meixell GE, Bristow MR, Canver CC. Angiotensin-(1-7) formation in the intact human heart in vivo dependence on angiotensin II as substrate. Circulation. 2003; 108(14): 1679-1681.
44. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circulation Research. 2000; 87(5): e1-e9.
45. Zisman LS, Keller RS, Weaver B, Lin Q, Speth R, Bristow MR, et al. Increased angiotensin-(1-7)-forming activity in failing human heart ventricles: Evidence for upregulation of the angiotensin-converting enzyme homologue ACE2. Circulation. 2003; 108(14): 1707-1712.
46. Burrell LM, Risvanis J, Kubota E, Dean RG, MacDonald PS, Lu S, et al. Myocardial infarction increases ACE2 expression in rat and humans. European Heart Journal. 2005; 26(4): 369-375.
47. Santos RA, Maric C, Silva DM, Machado RP, de Buhr I, Heringer-Walther S, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. Proceedings of the National Academy of Sciences. 2003; 100(14): 8258-8263.
48. Mercure C, Yogi A, Callera GE, Aranha AB, Bader M, Ferreira AJ, et al. Angiotensin (1-7) blunts hypertensive cardiac remodeling by a direct effect on the heart. Circulation Research. 2008; 103(11): 1319-1326.
49. Grobe JL, Mecca AP, Mao H, Katovich MJ. Chronic angiotensin-(1-7) prevents cardiac fibrosis in DOCA-salt model of hypertension. American Journal of Physiology-Heart and Circulatory Physiology. 2006; 290(6): H2417-H2423.

50. Flores-Muñoz M, Godinho BM, Almalik A, Nicklin SA. Adenoviral delivery of angiotensin-(1-7) or angiotensin-(1-9) inhibits cardiomyocyte hypertrophy via the Mas or angiotensin type 2 receptor. PloS one. 2012; 7(9): e45564.
51. Wilsdorf T, Gainer JV, Murphey LJ, Vaughan DE, Brown NJ. Angiotensin-(1-7) does not affect vasodilator or TPA responses to bradykinin in human forearm. Hypertension. 2001; 37(4): 1136-1140.
52. Davie AP, McMurray JJ. Effect of angiotensin-(1-7) and bradykinin in patients with heart failure treated with an ACE inhibitor. Hypertension. 1999; 34(3): 457-460.
53. Laragh JH. Hormones and the pathogenesis of congestive heart failure: vasopressin, aldosterone, and angiotensin II: further evidence for renal-adrenal interaction from studies in hypertension and in cirrhosis. Circulation. 1962; 25(6): 1015-1023.
54. Nicholls M, Espiner E, Donald R, Hughes H. Aldosterone and its regulation during diuresis in patients with gross congestive heart failure. Clinical Science. 1974; 47(4): 301-315.
55. Swedberg K, Eneroth P, Kjekshus J, Wilhelmsen L. Hormones regulating cardiovascular function in patients with severe congestive heart failure and their relation to mortality. CONSENSUS Trial Study Group. Circulation. 1990; 82(5): 1730-1736.
56. Cohn JN, Anand IS, Latini R, Masson S, Chiang YT, Glazer R, et al. Sustained reduction of aldosterone in response to the angiotensin receptor blocker valsartan in patients with chronic heart failure: results from the Valsartan Heart Failure trial. Circulation. 2003; 108(11): 1306-1309.
57. Bayliss J, Norell M, Canepa-Anson R, Sutton G, Poole-Wilson P. Untreated heart failure: clinical and neuroendocrine effects of introducing diuretics. British Heart Journal. 1987; 57(1): 17-22.
58. Anand I, Ferrari R, Kalra G, Wahi P, Poole-Wilson P, Harris P. Edema of cardiac origin. Studies of body water and sodium, renal function, hemodynamic indexes, and plasma hormones in untreated congestive cardiac failure. Circulation. 1989; 80(2): 299-305.
59. Dzau VJ, Colucci WS, Hollenberg N, Williams G. Relation of the renin-angiotensin-aldosterone system to clinical state in congestive heart failure. Circulation. 1981; 63(3): 645-651.
60. Kubo SH, Clark M, Laragh JH, Borer JS, Cody RJ. Identification of normal neurohormonal activity in mild congestive heart failure and stimulating effect of upright posture and diuretics. American Journal of Cardiology. 1987; 60(16): 1322-1328.
61. Francis G, Benedict C, Johnstone D, Kirlin P, Nicklas J, Liang CS, et al. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. A substudy of the Studies of Left Ventricular Dysfunction (SOLVD). Circulation. 1990; 82(5): 1724-1729.
62. Camargo C, Dowdy A, Hancock E, Luetscher J. Decreased plasma clearance and hepatic extraction of aldosterone in patients with heart failure. Journal of Clinical Investigation. 1965; 44(3): 356-365.
63. Tait J, Bougas J, Little B, Tait S, Flood C. Splanchnic extraction and clearance of aldosterone in subjects with minimal and marked cardiac dysfunction. Journal of Clinical Endocrinology and Metabolism. 1965; 25(2): 219-228.
64. Weber KT. Aldosterone in congestive heart failure. New England Journal of Medicine. 2001; 345(23): 1689-1697.
65. Epstein FH, Schrier RW, Abraham WT. Hormones and hemodynamics in heart failure. New England Journal of Medicine. 1999; 341(8): 577-585.
66. Funder JW. RALES, EPHESUS and redox. Journal of Steroid Biochemistry and Molecular Biology. 2005; 93(2): 121-125.

67. Funder JW. Reconsidering the roles of the mineralocorticoid receptor. Hypertension. 2009; 53(2): 286-290.
68. Müller J. Aldosterone: the minority hormone of the adrenal cortex. Steroids. 1995; 60(1): 2-9.
69. Connolly C, Wills M. Plasma cortisol levels in heart failure. British Medical Journal. 1967; 2(5543): 25-26.
70. Connolly C, Wills M. Plasma cortisol levels in right and left ventricular failure. Journal of Clinical Pathology. 1969; 22(5): 598-601.
71. Anker SD, Chua TP, Ponikowski P, Harrington D, Swan JW, Kox WJ, et al. Hormonal changes and catabolic/anabolic imbalance in chronic heart failure and their importance for cardiac cachexia. Circulation. 1997; 96(2): 526-534.
72. Güder G, Bauersachs J, Frantz S, Weismann D, Allolio B, Ertl G, et al. Complementary and incremental mortality risk prediction by cortisol and aldosterone in chronic heart failure. Circulation. 2007; 115(13): 1754-1761.
73. Yamaji M, Tsutamoto T, Kawahara C, Nishiyama K, Yamamoto T, Fujii M, et al. Serum cortisol as a useful predictor of cardiac events in patients with chronic heart failure: the impact of oxidative stress. Circulation Heart Failure. 2009; 2(6): 608-615.
74. Gwynne JT, Strauss JF. The role of lipoproteins in steroidogenesis and cholesterol metabolism in steroidogenic glands. Endocrine Reviews. 1982; 3(3): 299-329.
75. Stocco DM, Clark BJ. Role of the steroidogenic acute regulatory protein (StAR) in steroidogenesis. Biochemical Pharmacology. 1996; 51(3): 197-205.
76. White PC, New MI, Dupont B. Structure of human steroid 21-hydroxylase genes. Proceedings of the National Academy of Sciences. 1986; 83(14): 5111-5115.
77. Boon W, Coghlan J, Curnow K, McDougall JG. Aldosterone secretion: a molecular perspective. Trends in Endocrinology and Metabolism. 1997; 8(9): 346-354.
78. Mornet E, Dupont J, Vitek A, White P. Characterization of two genes encoding human steroid 11 beta-hydroxylase. Journal of Biological Chemistry. 1989; 264(35): 20961-20967.
79. Albiston AL, Obeyesekere VR, Smith RE, Krozowski ZS. Cloning and tissue distribution of the human 11beta-hydroxysteroid dehydrogenase type 2 enzyme. Molecular and Cellular Endocrinology. 1994; 105(2): R11-R17.
80. Bocchi B, Fagart J, Cluzeaud F, Fay M, Rafestin-Oblin ME, Farman N. Glucocorticoid metabolism by 11beta- hydroxysteroid dehydrogenase type 2 modulates human mineralocorticoid receptor transactivation activity. Journal of Steroid Biochemistry and Molecular Biology. 2003; 84(2): 239-244.
81. Seckl JR, Walker BR. Minireview: 11beta-hydroxysteroid dehydrogenase type 1—a tissue-specific amplifier of glucocorticoid action. Endocrinology. 2001; 142(4): 1371-1376.
82. Bujalska IJ, Walker EA, Tomlinson JW, Hewison M, Stewart PM. 11beta-hydroxysteroid dehydrogenase type 1 in differentiating omental human preadipocytes: from de-activation to generation of cortisol. Endocrine Research. 2002; 28(4): 449-461.
83. Oelkers W, Brown JJ, Fraser R, Lever AF, Morton JJ, Robertson JI. Sensitization of the adrenal cortex to angiotensin II in sodium-deplete man. Circulation Research. 1974; 34(1): 69-77.
84. Hollenberg NK, Chenitz WR, Adams DF, Williams GH. Reciprocal influence of salt intake on adrenal glomerulosa and renal vascular responses to angiotensin II in normal man. Journal of Clinical Investigation. 1974; 54(1): 34-42.
85. Connell JM, Davies E. The new biology of aldosterone. Journal of Endocrinology. 2005;

186(1): 1-20.

86. Tian Y, Balla T, Baukal AJ, Catt KJ. Growth responses to angiotensin II in bovine adrenal glomerulosa cells. American Journal of Physiology-Endocrinology And Metabolism. 1995; 268(1): E135-E144.
87. Otis M, Campbell S, Payet MD, Gallo-Payet N. The growth-promoting effects of angiotensin II in adrenal glomerulosa cells: an interactive tale. Molecular and Cellular Endocrinology. 2007; 273(1): 1-5.
88. Sugiyama T, Yoshimoto T, Tsuchiya K, Gochou N, Hirono Y, Tateno T, et al. Aldosterone induces angiotensin converting enzyme gene expression via a JAK2-dependent pathway in rat endothelial cells. Endocrinology. 2005; 146(9): 3900-3906.
89. Ullian ME, Schelling JR, Linas SL. Aldosterone enhances angiotensin II receptor binding and inositol phosphate responses. Hypertension. 1992; 20(1): 67-73.
90. Keidar S, Kaplan M, Pavlotzky E, Coleman R, Hayek T, Hamoud S, et al. Aldosterone administration to mice stimulates macrophage NADPH oxidase and increases atherosclerosis development: a possible role for angiotensin-converting enzyme and the receptors for angiotensin II and aldosterone. Circulation. 2004; 109(18): 2213-2220.
91. Ardaillou R, Chansel D. Synthesis and effects of active fragments of angiotensin II. Kidney International. 1997; 52(6): 1458-1468.
92. Troyanovskaya M, Song L, Jayaraman G, Healy DP. Expression of aminopeptidase A, an angiotensinase, in glomerular mesangial cells. Hypertension. 1996; 27(3): 518-522.
93. Braley LM, Menachery AI, Underwood RH, Williams GH. Is the adrenal angiotensin receptor angiotensin II—or angiotensin III like? Acta Endocrinologica. 1983; 102(1): 116-121.
94. Kono T, Oseko F, Shimpo S, Nanno M, Endo J. Biological activity of des-asp1-angiotensin II (angiotensin III) in man. Journal of Clinical Endocrinology and Metabolism. 1975; 41(6): 1174-1177.
95. Semple PF, Nicholls MG, Tree M, Fraser R. [des-Asp1] angiotensin II in the dog: blood levels and effect on aldosterone. Endocrinology. 1978; 103(4): 1476-1482.
96. Yatabe J, Yoneda M, Yatabe MS, Watanabe T, Felder RA, Jose PA, et al. Angiotensin III stimulates aldosterone secretion from adrenal gland partially via angiotensin II type 2 receptor but not angiotensin II type 1 receptor. Endocrinology. 2011; 152(4): 1582-1588.
97. Pralong WF, Hunyady L, Varnai P, Wollheim CB, Spät A. Pyridine nucleotide redox state parallels production of aldosterone in potassium-stimulated adrenal glomerulosa cells. Proceedings of the National Academy of Sciences. 1992; 89(1): 132-136.
98. Himathongkam T, Dluhy RG, WIilliams GH. Potassium-aldosterone-renin interrelationships. Journal of Clinical Endocrinology and Metabolism. 1975; 41(1): 153-159.
99. Fredulund P, Saltman S, Kondo T, Douglas J, Catt KJ. Aldosterone production by isolated glomerulosa cells: modulation of sensitivity to angiotensin II and ACTH by extracellular potassium concentration. Endocrinology. 1977; 100(2): 481-486.
100. Young DB, Smith M, Jackson TE, Scott RE. Multiplicative interaction between angiotensin II and K concentration in stimulation of aldosterone. American Journal of Physiology-Endocrinology And Metabolism. 1984; 247(3): E328-E335.
101. Spät A. Glomerulosa cell—a unique sensor of extracellular K^+ concentration. Molecular and Cellular Endocrinology. 2004; 217(1): 23-26.
102. Dluhy RG, Axelrod L, Underwood RH, Williams GH. Studies of the control of plasma aldosterone concentration in normal man: II. Effect of dietary potassium and acute potassium infusion. Journal of Clinical Investigation. 1972; 51(8): 1950-1957.

103. Tremblay A, Parker K, Lehoux J. Dietary potassium supplementation and sodium restriction stimulate aldosterone synthase but not 11 beta-hydroxylase P-450 messenger ribonucleic acid accumulation in rat adrenals and require angiotensin II production. Endocrinology. 1992; 130(6): 3152-3158.
104. Yagci A, Oertle M, Seiler H, Schmid D, Campofranco C, Müller J. Potassium induces multiple steroidogenic enzymes in cultured rat zona glomerulosa cells. Endocrinology. 1996; 137(6): 2406-2414.
105. Allen RG, Carey C, Parker JD, Mortrud MT, Mellon SH, Low MJ. Targeted ablation of pituitary pre-proopiomelanocortin cells by herpes-simplex virus-1 thymidine kinase differentially regulates messenger-rnas encoding the adrenocorticotropin receptor and aldosterone synthase in the mouse adrenal-gland. Molecular Endocrinology. 1995; 9(8): 1005-1016.
106. Arvat E, Di Vito L, Lanfranco F, Maccario M, Baffoni C, Rossetto R, et al. Stimulatory effect of adrenocorticotropin on cortisol, aldosterone, and dehydroepiandrosterone secretion in normal humans: dose-response study. Journal of Clinical Endocrinology and Metabolism. 2000; 85(9): 3141-3146.
107. Oelkers W, Harendt H, Exner P. Prolonged ACTH infusion suppresses aldosterone secretion in spite of high renin activity. Acta Endocrinologica. 1985; 108(1): 91-97.
108. Aguilera G, Kiss A, Lu A, Camacho C. Regulation of adrenal steroidogenesis during chronic stress. Endocrine Research. 1996; 22(4): 433-443.
109. Fuchs-Hammoser R, Schweiger M, Oelkers W. The effect of chronic low-dose infusion of ACTH (1-24) on renin, renin-substrate, aldosterone and other corticosteroids in sodium replete and deplete man. Acta Endocrinologica. 1980; 95(2): 198-206.
110. McDougal JG, Butkus A, Coghlan JP, Denton DA, Müller J, Oddie CJ, et al. Biosynthetic and morphological evidence for inhibition of aldosterone production following administration of ACTH to sheep. Acta Endocrinologica. 1980; 94(4): 559-570.
111. Vinson G. Adrenocortical zonation and ACTH. Microscopy Research and Technique. 2003; 61(3): 227-239.
112. Abayasekara D, Vazir H, Whitehouse B, Price G, Hinson J, Vinson G. Studies on the mechanisms of ACTH-induced inhibition of aldosterone biosynthesis in the rat adrenal cortex. Journal of Endocrinology. 1989; 122(3): 625-632.
113. Seely EW, Conlin PR, Brent GA, Dluhy RG. Adrenocorticotropin stimulation of aldosterone: prolonged continuous versus pulsatile infusion. Journal of Clinical Endocrinology and Metabolism. 1989; 69(5): 1028-1032.
114. Williams GH, Rose LI, Dluhy RG, Dingman JF, Lauler DP. Aldosterone response to sodium restriction and ACTH stimulation in panhypopituitarism. Journal of Clinical Endocrinology and Metabolism. 1971; 32(1): 27-35.
115. Armbruster H, Vetter W, Beckerhoff R, Nussberger J, Vetter H, Siegenthaler W. Diurnal variations of plasma aldosterone in supine man: relationship to plasma renin activity and plasma cortisol. Acta Endocrinologica. 1975; 80(1): 95-103.
116. Ganguly A, Hampton T. Calcium-dependence of serotonin-mediated aldosterone secretion and differential effects of calcium antagonists. Life Sciences. 1985; 36(15): 1459-1464.
117. Lefebvre H, Contesse V, Delarue C, Legrand A, Kuhn J, Vaudry H, et al. The serotonin-4 receptor agonist cisapride and angiotensin-II exert additive effects on aldosterone secretion in normal man. Journal of Clinical Endocrinology and Metabolism. 1995; 80(2): 504-507.
118. Zeng Z, Naruse M, Guan BJ, Naruse K, Sun M, Zang M, et al. Endothelin stimulates

aldosterone secretion in vitro from normal adrenocortical tissue, but not adenoma tissue, in primary aldosteronism. Journal of Clinical Endocrinology and Metabolism. 1992; 74(4): 874-878.

119. Woodcock EA, Tanner JK, Caroccia LM, Little PJ. Mechanisms involved in the stimulation of aldosterone production by angiotensin II, vasopressin and endothelin. Clinical and Experimental Pharmacology and Physiology. 1990; 17(4): 263-267.
120. Goodfriend T, Ball D, Gardner H. An oxidized derivative of linoleic acid affects aldosterone secretion by adrenal cells in vitro. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2002; 67(2): 163-167.
121. Goodfriend TL, Ball DL, Egan BM, Campbell WB, Nithipatikom K. Epoxy-keto derivative of linoleic acid stimulates aldosterone secretion. Hypertension. 2004; 43(2): 358-363.
122. Ehrhart-Bornstein M, Lamounier-Zepter V, Schraven A, Langenbach J, Willenberg H, Barthel A, et al. Human adipocytes secrete mineralocorticoid-releasing factors. Proceedings of the National Academy of Sciences. 2003; 100(24): 14211-14216.
123. Laragh JH. Atrial natriuretic hormone, the renin-aldosterone axis, and blood pressure-electrolyte homeostasis. New England Journal of Medicine. 1985; 313(21): 1330-1340.
124. De Bold A, Borenstein H, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sciences. 1981; 28(1): 89-94.
125. McMurray J, Coutie W, McFarlane L, Struthers A. Atrial natriuretic factor inhibits ACTH stimulated aldosterone, but not cortisol, secretion in man. European Journal of Clinical Pharmacology. 1988; 35(4): 409-412.
126. Oelkers W, Kleiner S, Bähr V. Effects of incremental infusions of atrial natriuretic factor on aldosterone, renin, and blood pressure in humans. Hypertension. 1988; 12(4): 462-467.
127. Struthers A, Anderson J, Payne N, Causon R, Slater JDH, Bloom S. The effect of atrial natriuretic peptide on plasma renin activity, plasma aldosterone, and urinary dopamine in man. European Journal of Clinical Pharmacology. 1986; 31(2): 223-226.
128. Johnston CI, Hodsman PG, Kohzuki M, Casley DJ, Fabris B, Phillips PA. Interaction between atrial natriuretic peptide and the renin angiotensin aldosterone system. Endogenous antagonists. American Journal of Medicine. 1989; 87(6): 24S-28S.
129. Waldman SA, Rapoport R, Murad F. Atrial natriuretic factor selectively activates particulate guanylate cyclase and elevates cyclic GMP in rat tissues. Journal of Biological Chemistry. 1984; 259(23): 14332-14334.
130. Kitamura K, Sakata J, Kangawa K, Kojima M, Matsuo H, Eto T, et al. Cloning and characterization of cDNA encoding a precursor for human adrenomedullin. Biochemical and Biophysical Research Communications. 1993; 194(2): 720-725.
131. Sugo S, Minamino N, Kangawa K, Miyamoto K, Kitamura K, Sakata J, et al. Endothelial cells actively synthesize and secrete adrenomedullin. Biochemical and Biophysical Research Communications. 1994; 201(3): 1160-1166.
132. Andreis P, Neri G, Prayer-Galetti T, Rossi G, Gottardo G, Malendowicz L, et al. Effects of adrenomedullin on the human adrenal glands: an in vitro study. Journal of Clinical Endocrinology and Metabolism. 1997; 82(4): 1167-1170.
133. Yamaguchi T, Baba K, Doi Y, Yano K. Effect of adrenomedullin on aldosterone secretion by dispersed rat adrenal zona glomerulosa cells. Life Sciences. 1994; 56(6): 379-387.
134. Yamaguchi T, Baba K, Doi Y, Yano K, Kitamura K, Eto T. Inhibition of aldosterone production by adrenomedullin, a hypotensive peptide, in the rat. Hypertension. 1996;

28(2): 308-314.

135. Petrie MC, Hillier C, Morton JJ, McMurray JJ. Adrenomedullin selectively inhibits angiotensin II-induced aldosterone secretion in humans. Journal of Hypertension. 2000; 18(1): 61-64.
136. Lundblad JR, Roberts JL. Regulation of proopiomelanocortin gene expression in pituitary. Endocrine Reviews. 1988; 9(1): 135-158.
137. Taylor AL, Fishman LM. Corticotropin-releasing hormone. New England Journal of Medicine. 1988; 319(4): 213-222.
138. Boutillier A, Monnier D, Lorang D, Lundblad J, Roberts J, Loeffler J. Corticotropin-releasing hormone stimulates proopiomelanocortin transcription by cFos-dependent and-independent pathways: characterization of an AP1 site in exon 1. Molecular Endocrinology. 1995; 9(6): 745-755.
139. Luo X, Kiss A, Makara G, Lolait SJ, Aguilera G. Stress-specific regulation of corticotropin releasing hormone receptor expression in the paraventricular and supraoptic nuclei of the hypothalamus in the rat. Journal of Neuroendocrinology. 2006; 6(6): 689-696.
140. DeBold CR, Sheldon WR, DeCherney GS, Jackson RV, Alexander AN, Vale W, et al. Arginine vasopressin potentiates adrenocorticotropin release induced by ovine corticotropin-releasing factor. Journal of Clinical Investigation. 1984; 73(2): 533-538.
141. Widmaier EP, Plotsky PM, Sutton SW, Vale WW. Regulation of corticotropin-releasing factor secretion in vitro by glucose. American Journal of Physiology-Endocrinology And Metabolism. 1988; 255(3): E287-E292.
142. Watabe T, Tanaka K, Kumagae M, Itoh S, Takeda F, Morio K, et al. Hormonal responses to insulin-induced hypoglycemia in man. Journal of Clinical Endocrinology and Metabolism. 1987; 65(6): 1187-1191.
143. Drouin J, Charron J, Gagner JP, Jeannotte L, Nemer M, Plante RK, et al. Pro-opiomelanocortin gene: A model for negative regulation of transcription by glucocorticoids. Journal of Cellular Biochemistry. 1987; 35(4): 293-304.
144. Luo X, Kiss A, Rabadan-Diehl C, Aguilera G. Regulation of hypothalamic and pituitary corticotropin-releasing hormone receptor messenger ribonucleic acid by adrenalectomy and glucocorticoids. Endocrinology. 1995; 136(9): 3877-3883.
145. Veldhuis JD, Iranmanesh A, Lizarralde G, Johnson ML. Amplitude modulation of a burstlike mode of cortisol secretion subserves the circadian glucocorticoid rhythm. American Journal of Physiology-Endocrinology And Metabolism. 1989; 257(1):. E6-E14.
146. Veldhuis JD, Iranmanesh A, Johnson ML, Lizarralde G, others . Amplitude, but not frequency, modulation of adrenocorticotropin secretory bursts gives rise to the nyctohemeral rhythm of the corticotropic axis in man. Journal of Clinical Endocrinology and Metabolism. 1990; 71(2): 452-463.
147. Horisberger JD. Amiloride-sensitive Na channels. Current Opinion in Cell Biology. 1998; 10(4): 443-449.
148. Ergonul Z, Frindt G, Palmer LG. Regulation of maturation and processing of ENaC subunits in the rat kidney. American Journal of Physiology-Renal Physiology. 2006; 291(3): F683-F693.
149. Kemendy AE, Kleyman TR, Eaton DC. Aldosterone alters the open probability of amiloride-blockable sodium channels in A6 epithelia. American Journal of Physiology-Cell Physiology. 1992; 263(4): C825-C837.
150. Bastl CP, Hayslett JP. The cellular action of aldosterone in target epithelia. Kidney International. 1992; 42(2): 250-264.

151. Kim GH, Masilamani S, Turner R, Mitchell C, Wade JB, Knepper MA. The thiazide-sensitive Na-Cl cotransporter is an aldosterone-induced protein. Proceedings of the National Academy of Sciences. 1998; 95(24): 14552-14557.
152. Garg LC, Narang N. Effects of aldosterone on NEM-sensitive ATPase in rabbit nephron segments. Kidney International. 1988; 34(1): 13-17.
153. Juurlink DN, Mamdani MM, Lee DS, Kopp A, Austin PC, Laupacis A, et al. Rates of hyperkalemia after publication of the Randomized Aldactone Evaluation Study. New England Journal of Medicine. 2004; 351(6): 543-551.
154. Pascual-Le Tallec L, Lombès M. The mineralocorticoid receptor: a journey exploring its diversity and specificity of action. Molecular Endocrinology. 2005; 19(9): 2211-2221.
155. Arriza JL, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housman DE, et al. Cloning of human mineralocorticoid receptor complementary DNA: Structural and functional kinship with the glucocorticoid receptor. Science. 1987; 237(4812): 268-275.
156. Funder JW, Pearce PT, Smith R, Smith AI. Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. Science. 1988; 242(4878): 583-585.
157. Rogerson FM, Brennan FE, Fuller PJ. Mineralocorticoid receptor binding, structure and function. Molecular and Cellular Endocrinology. 2004; 217(1): 203-212.
158. Bhargava A, Fullerton MJ, Myles K, Purdy TM, Funder JW, Pearce D, et al. The serum-and glucocorticoid-induced kinase is a physiological mediator of aldosterone action. Endocrinology. 2001; 142(4): 1587-1594.
159. Brennan FE, Fuller PJ. Rapid upregulation of serum and glucocorticoid-regulated kinase (sgk) gene expression by corticosteroids in vivo. Molecular and Cellular Endocrinology. 2000; 166(2): 129-136.
160. Boyd C, Náray-Fejes-Tóth A. Gene regulation of ENaC subunits by serum-and glucocorticoid-inducible kinase-1. American Journal of Physiology-Renal Physiology. 2005; 288(3): F505-F512.
161. McCormick JA, Bhalla V, Pao AC, Pearce D. SGK1: a rapid aldosterone-induced regulator of renal sodium reabsorption. Physiology. 2005; 20(2): 134-139.
162. Rotin D. Regulation of the epithelial sodium channel (ENaC) by accessory proteins. Current Opinion in Nephrology and Hypertension. 2000; 9(5): 529-534.
163. Yoo D, Kim BY, Campo C, Nance L, King A, Maouyo D, et al. Cell surface expression of the ROMK (Kir 1.1) channel is regulated by the aldosterone-induced kinase, SGK-1, and protein kinase A. Journal of Biological Chemistry. 2003; 278(25): 23066-23075.
164. Alzamora R, Marusic ET, Gonzalez M, Michea L. Nongenomic effect of aldosterone on Na+,K+-adenosine triphophatase in arterial vessels. Endocrinology. 2003; 144(4): 1266-1272.
165. Mihailidou AS, Mardini M, Funder JW. Rapid, nongenomic effects of aldosterone in the heart mediated by ε protein kinase C. Endocrinology. 2004; 145(2): 773-780.
166. Boldyreff B, Wehling M. Rapid aldosterone actions: from the membrane to signaling cascades to gene transcription and physiological effects. Journal of Steroid Biochemistry and Molecular Biology. 2003; 85(2): 375-381.
167. Chai W, Garrelds IM, de Vries R, Batenburg WW, van Kats JP, Danser AHJ. Nongenomic effects of aldosterone in the human heart interaction with angiotensin II. Hypertension. 2005; 46(4): 701-706.
168. Alzamora R, Michea L, Marusic ET. Role of 11beta-hydroxysteroid dehydrogenase in nongenomic aldosterone effects in human arteries. Hypertension. 2000; 35(5): 1099-1104.
169. Oberleithner H, Weigt M, Westphale H, Wang W. Aldosterone activates Na+/H+

exchange and raises cytoplasmic pH in target cells of the amphibian kidney. Proceedings of the National Academy of Sciences. 1987; 84(5): 1464-1468.

170. Estrada M, Liberona JL, Miranda M, Jaimovich E. Aldosterone-and testosterone-mediated intracellular calcium response in skeletal muscle cell cultures. American Journal of Physiology-Endocrinology And Metabolism. 2000; 279(1): E132-E139.
171. Wehling M, Käsmayr J, Theisen K. Aldosterone influences free intracellular calcium in human mononuclear leukocytes in vitro. Cell Calcium. 1990; 11(9): 565-571.
172. Klein K, Henk W. Clinical experimental studies on the influence of aldosterone on hemodynamics and blod coagulation. Zeitschrift für Kreislaufforschung. 1963; 52: 40-53.
173. Wehling M, Spes CH, Win N, Janson CP, Schmidt BMW, Theisen K, et al. Rapid cardiovascular action of aldosterone in man. Journal of Clinical Endocrinology and Metabolism. 1998; 83(10): 3517-3522.
174. Schmidt BMW, Georgens AC, Martin N, Tillmann HC, Feuring M, Christ M, et al. Interaction of rapid nongenomic cardiovascular aldosterone effects with the adrenergic system. Journal of Clinical Endocrinology and Metabolism. 2001; 86(2): 761-767.
175. Yee K, Struthers A. Aldosterone blunts the baroreflex response in man. Clinical Science. 1998; 95: 687-692.
176. MacFadyen RJ, Barr CS, Struthers AD. Aldosterone blockade reduces vascular collagen turnover, improves heart rate variability and reduces early morning rise in heart rate in heart failure patients. Cardiovascular Research. 1997; 35(1): 30-34.
177. Lösel RM, Feuring M, Falkenstein E, Wehling M. Nongenomic effects of aldosterone: cellular aspects and clinical implications. Steroids. 2002; 67(6): 493-498.
178. Michea L, Delpiano AM, Hitschfeld C, Lobos L, Lavandero S, Marusic ET. Eplerenone blocks nongenomic effects of aldosterone on the Na+/H+ exchanger, intracellular Ca2+ levels, and vasoconstriction in mesenteric resistance vessels. Endocrinology. 2005; 146(3): 973-980.
179. Fujita M, Minamino T, Asanuma H, Sanada S, Hirata A, Wakeno M, et al. Aldosterone nongenomically worsens ischemia via protein kinase C-dependent pathways in hypoperfused canine hearts. Hypertension. 2005; 46(1): 113-117.
180. Thomas P, Pang Y, Filardo E, Dong J. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. Endocrinology. 2005; 146(2): 624-632.
181. Funder JW. Aldosterone and the cardiovascular system: genomic and nongenomic effects. Endocrinology. 2006; 147(12): 5564-5567.
182. Lombes M, Oblin M, Gasc J, Baulieu E, Farman N, Bonvalet J. Immunohistochemical and biochemical evidence for a cardiovascular mineralocorticoid receptor. Circulation Research. 1992; 71(3): 503-510.
183. Edwards C, Burt D, McIntyre M, De Kloet E, Stewart P, Brett L, et al. Localisation of 11beta-hydroxysteroid dehydrogenase—tissue specific protector of the mineralocorticoid receptor. Lancet. 1988; 332(8618): 986-989.
184. Roland B, Funder J. Localization of 11beta-hydroxysteroid dehydrogenase type 2 in rat tissues: in situ studies. Endocrinology. 1996; 137(3): 1123-1128.
185. Romero DG, Zhou MY, Gomez-Sanchez CE. Cloning and expression of the bovine 11beta-hydroxysteroid dehydrogenase type-2. Journal of Steroid Biochemistry and Molecular Biology. 2000; 72(5): 231-237.
186. Farman N, Bocchi B. Mineralocorticoid selectivity: molecular and cellular aspects. Kidney international. 2000; 57(4): 1364-1369.

187. Hatakeyama H, Inaba S, Miyamori I. 11beta-hydroxysteroid dehydrogenase in cultured human vascular cells possible role in the development of hypertension. Hypertension. 1999; 33(5): 1179-1184.
188. Hadoke PWF, Christy C, Kotelevtsev YV, Williams BC, Kenyon CJ, Seckl JR, et al. Endothelial cell dysfunction in mice after transgenic knockout of type 2, but not type 1, 11β-hydroxysteroid dehydrogenase. Circulation. 2001; 104(23): 2832-2837.
189. Callera GE, Touyz RM, Teixeira SA, Muscara MN, Carvalho MH, Fortes ZB, et al. ETA receptor blockade decreases vascular superoxide generation in DOCA-salt hypertension. Hypertension. 2003; 42(4): 811-817.
190. Callera GE, Touyz RM, Tostes RC, Yogi A, He Y, Malkinson S, et al. Aldosterone activates vascular p38MAP kinase and NADPH oxidase via c-Src. Hypertension. 2005; 45(4): 773-779.
191. Rajagopalan S, Kurz S, Münzel T, Tarpey M, Freeman BA, Griendling KK, et al. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. Journal of Clinical Investigation. 1996; 97(8): 1916-1923.
192. Zalba G, Beaumont FJ, San José G, Fortuño A, Fortuño MA, Etayo JC, et al. Vascular NADH/NADPH oxidase is involved in enhanced superoxide production in spontaneously hypertensive rats. Hypertension. 2000; 35(5): 1055-1061.
193. Miyata K, Rahman M, Shokoji T, Nagai Y, Zhang GX, Sun GP, et al. Aldosterone stimulates reactive oxygen species production through activation of NADPH oxidase in rat mesangial cells. Journal of the American Society of Nephrology. 2005; 16(10): 2906-2912.
194. Bonizzi G, Piette J, Schoonbroodt S, Greimers R, Havard L, Merville MP, et al. Reactive oxygen intermediate-dependent NF-kappa B activation by interleukin-1beta requires 5-lipoxygenase or NADPH oxidase activity. Molecular and Cellular Biology. 1999; 19(3): 1950-1960.
195. Canty Jr TG, Boyle Jr EM, Farr A, Morgan EN, Verrier ED, Pohlman TH. Oxidative stress induces NF-kappa B nuclear translocation without degradation of IκBα. Circulation. 1999; 100(suppl 2): II361-II364.
196. Sun B, Fan H, Honda T, Fujimaki R, Lafond-Walker A, Masui Y, et al. Activation of NF kappa B and expression of ICAM-1 in ischemic-reperfused canine myocardium. Journal of Molecular and Cellular Cardiology. 2001; 33(1): 109-119.
197. Sung FL, Zhu TY, Au-Yeung KKW, Siow YL, O K . Enhanced MCP-1 expression during ischemia/reperfusion injury is mediated by oxidative stress and NF-κB. Kidney International. 2002; 62(4): 1160-1170.
198. Rocha R, Chander PN, Khanna K, Zuckerman A, Stier CT. Mineralocorticoid blockade reduces vascular injury in stroke-prone hypertensive rats. Hypertension. 1998; 31(1): 451-458.
199. Sun Y, Zhang J, Lu L, Chen SS, Quinn MT, Weber KT. Aldosterone-induced inflammation in the rat heart: role of oxidative stress. American Journal of Pathology. 2002; 161(5): 1773-1781.
200. Young MJ, Moussa L, Dilley R, Funder JW. Early inflammatory responses in experimental cardiac hypertrophy and fibrosis: effects of 11beta-hydroxysteroid dehydrogenase inactivation. Endocrinology. 2003; 144(3): 1121-1125.
201. Ahokas RA, Sun Y, Bhattacharya SK, Gerling IC, Weber KT. Aldosteronism and a proinflammatory vascular phenotype. Circulation. 2005; 111(1): 51-57.
202. Weber KT. From inflammation to fibrosis: a stiff stretch of highway. Hypertension. 2004; 43(4): 716-719.

203. Virdis A, Neves MF, Amiri F, Viel E, Touyz RM, Schiffrin EL. Spironolactone improves angiotensin-induced vascular changes and oxidative stress. Hypertension. 2002; 40(4): 504-510.
204. Imanishi T, Ikejima H, Tsujioka H, Kuroi A, Kobayashi K, Muragaki Y, et al. Addition of eplerenone to an angiotensin-converting enzyme inhibitor effectively improves nitric oxide bioavailability. Hypertension. 2008; 51(3): 734-741.
205. Kuster GM, Kotlyar E, Rude MK, Siwik DA, Liao R, Colucci WS, et al. Mineralocorticoid receptor inhibition ameliorates the transition to myocardial failure and decreases oxidative stress and inflammation in mice with chronic pressure overload. Circulation. 2005; 111(4): 420-427.
206. Takebayashi K, Matsumoto S, Aso Y, Inukai T. Aldosterone blockade attenuates urinary monocyte chemoattractant protein-1 and oxidative stress in patients with type 2 diabetes complicated by diabetic nephropathy. Journal of Clinical Endocrinology and Metabolism. 2006; 91(6): 2214-2217.
207. Yamanari H, Nakamura K, Miura D, Yamanari S, Ohe T. Spironolactone and chlorthalidone in uncontrolled elderly hypertensive patients treated with calcium antagonists and angiotensin II receptor-blocker: effects on endothelial function, inflammation, and oxidative stress. Clinical and Experimental Hypertension. 2009; 31(7): 585-594.
208. Godfrey V, Farquharson CAJ, Macdonald JE, Yee KM, Struthers AD. Effect of spironolactone on C-reactive protein levels in patients with heart disease. International Journal of Cardiology. 2007; 117(2): 282-284.
209. Drexler H, Zeiher AM, Wollschlager H, Meinertz T, Just H, Bonzel T. Flow-dependent coronary artery dilatation in humans. Circulation. 1989; 80(3): 466-474.
210. Sinoway LI, Hendrickson C, Davidson W, Prophet S, Zelis R. Characteristics of flow-mediated brachial artery vasodilation in human subjects. Circulation Research. 1989; 64(1): 32-42.
211. Farquharson CAJ, Struthers AD. Aldosterone induces acute endothelial dysfunction in vivo in humans: evidence for an aldosterone-induced vasculopathy. Clinical Science. 2002; 103(4): 425-432.
212. Nagata D, Takahashi M, Sawai K, Tagami T, Usui T, Shimatsu A, et al. Molecular mechanism of the inhibitory effect of aldosterone on endothelial NO synthase activity. Hypertension. 2006; 48(1): 165-171.
213. Vásquez-Vivar J, Kalyanaraman B, Martásek P, Hogg N, Masters BSS, Karoui H, et al. Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. Proceedings of the National Academy of Sciences. 1998; 95(16): 9220-9225.
214. Leopold JA, Dam A, Maron BA, Scribner AW, Liao R, Handy DE, et al. Aldosterone impairs vascular reactivity by decreasing glucose-6-phosphate dehydrogenase activity. Nature Medicine. 2007; 13(2): 189-197.
215. Leopold JA, Zhang YY, Scribner AW, Stanton RC, Loscalzo J. Glucose-6-phosphate dehydrogenase overexpression decreases endothelial cell oxidant stress and increases bioavailable nitric oxide. Arteriosclerosis, Thrombosis, and Vascular Biology. 2003; 23(3): 411-417.
216. Oberleithner H. Aldosterone makes human endothelium stiff and vulnerable. Kidney International. 2005; 67(5): 1680-1682.
217. Sartório CL, Fraccarollo D, Galuppo P, Leutke M, Ertl G, Stefanon I, et al. Mineralocorticoid receptor blockade improves vasomotor dysfunction and vascular oxidative stress early after myocardial infarction. Hypertension. 2007; 50(5): 919-925.
218. Bauersachs J, Heck M, Fraccarollo D, Hildemann SK, Ertl G, Wehling M, et al. Addition

of spironolactone to angiotensin-converting enzyme inhibition in heart failure improves endothelial vasomotor dysfunction: role of vascular superoxide anion formation and endothelial nitric oxide synthase expression. Journal of the American College of Cardiology. 2002; 39(2): 351-358.

219. Nishizaka MK, Zaman MA, Green SA, Renfroe KY, Calhoun DA. Impaired endothelium-dependent flow-mediated vasodilation in hypertensive subjects with hyperaldosteronism. Circulation. 2004; 109(23): 2857-2861.
220. Farquharson CAJ, Struthers AD. Spironolactone increases nitric oxide bioactivity, improves endothelial vasodilator dysfunction, and suppresses vascular angiotensin I/angiotensin II conversion in patients with chronic heart failure. Circulation. 2000; 101(6): 594-597.
221. Macdonald J, Kennedy N, Struthers A. Effects of spironolactone on endothelial function, vascular angiotensin converting enzyme activity, and other prognostic markers in patients with mild heart failure already taking optimal treatment. Heart. 2004; 90(7): 765-770.
222. Brilla C, Weber K. Mineralocorticoid excess, dietary sodium, and myocardial fibrosis. Journal of Laboratory and Clinical Medicine. 1992; 120(6): 893-901.
223. Young M, Head G, Funder J. Determinants of cardiac fibrosis in experimental hypermineralocorticoid states. American Journal of Physiology-Endocrinology And Metabolism. 1995; 269(4): E657-E662.
224. Brilla CG, Zhou G, Matsubara L, Weber KT. Collagen metabolism in cultured adult rat cardiac fibroblasts: response to angiotensin II and aldosterone. Journal of Molecular and Cellular Cardiology. 1994; 26(7): 809-820.
225. Zhou G, Kandala JC, Tyagi SC, Katwa LC, Weber KT. Effects of angiotensin II and aldosterone on collagen gene expression and protein turnover in cardiac fibroblasts. Molecular and Cellular Biochemistry. 1996; 154(2): 171-178.
226. Fullerton MJ, Funder JW. Aldosterone and cardiac fibrosis: in vitro studies. Cardiovascular Research. 1994; 28(12): 1863-1867.
227. Kohler E, Bertschin S, Woodtli T, Resink T, Erne P. Does aldosterone-induced cardiac fibrosis involve direct effects on cardiac fibroblasts? Journal of Vascular Research. 1996; 33(4): 315-326.
228. Neumann S, Huse K, Semrau R, Diegeler A, Gebhardt R, Buniatian GH, et al. Aldosterone and D-glucose stimulate the proliferation of human cardiac myofibroblasts in vitro. Hypertension. 2002; 39(3): 756-760.
229. Rocha R, Rudolph AE, Frierdich GE, Nachowiak DA, Kekec BK, Blomme EA, et al. Aldosterone induces a vascular inflammatory phenotype in the rat heart. American Journal of Physiology-Heart and Circulatory Physiology. 2002; 283(5): H1802-H1810.
230. Rickard AJ, Morgan J, Tesch G, Funder JW, Fuller PJ, Young MJ. Deletion of mineralocorticoid receptors from macrophages protects against deoxycorticosterone/salt-induced cardiac fibrosis and increased blood pressure. Hypertension. 2009; 54(3): 537-543.
231. Caprio M, Newfell BG, la Sala A, Baur W, Fabbri A, Rosano G, et al. Functional mineralocorticoid receptors in human vascular endothelial cells regulate intercellular adhesion molecule-1 expression and promote leukocyte adhesion. Circulation Research. 2008; 102(11): 1359-1367.
232. Ouvrard-Pascaud A, Sainte-Marie Y, Bénitah JP, Perrier R, Soukaseum C, Nguyen Dinh Cat A, et al. Conditional mineralocorticoid receptor expression in the heart leads to life-threatening arrhythmias. Circulation. 2005; 111(23): 3025-3033.
233. Ammarguellat F, Larouche I, Schiffrin EL. Myocardial fibrosis in DOCA-salt

hypertensive rats: effect of endothelin ETA receptor antagonism. Circulation. 2001; 103(2): 319-324.

234. Ishimaru K, Ueno H, Kagitani S, Takabayashi D, Takata M, Inoue H. Fasudil attenuates myocardial fibrosis in association with inhibition of monocyte/macrophage infiltration in the heart of DOCA/salt hypertensive rats. Journal of Cardiovascular Pharmacology. 2007; 50(2): 187-194.
235. Kagitani S, Ueno H, Hirade S, Takahashi T, Takata M, Inoue H. Tranilast attenuates myocardial fibrosis in association with suppression of monocyte/macrophage infiltration in DOCA/salt hypertensive rats. Journal of Hypertension. 2004; 22(5): 1007-1015.
236. Kuwahara F, Kai H, Tokuda K, Takeya M, Takeshita A, Egashira K, et al. Hypertensive myocardial fibrosis and diastolic dysfunction: another model of inflammation? Hypertension. 2004; 43(4): 739-745.
237. Benetos A, Lacolley P, Safar M. Prevention of aortic fibrosis by spironolactone in spontaneously hypertensive rats. Arteriosclerosis, Thrombosis, and Vascular Biology. 1997; 17(6): 1152-1156.
238. Delyani JA, Robinson EL, Rudolph AE. Effect of a selective aldosterone receptor antagonist in myocardial infarction. American Journal of Physiology-Heart and Circulatory Physiology. 2001; 281(2): H647-H654.
239. Fraccarollo D, Galuppo P, Hildemann S, Christ M, Ertl G, Bauersachs J. Additive improvement of left ventricular remodeling and neurohormonal activation by aldosterone receptor blockade with eplerenone and ACE inhibition in rats with myocardial infarction. Journal of the American College of Cardiology. 2003; 42(9): 1666-1673.
240. Berry C, Murphy N, De Vito G, Galloway S, Seed A, Fisher C, et al. Effects of aldosterone receptor blockade in patients with mild-moderate heart failure taking a beta-blocker. European Journal of Heart Failure. 2007; 9(4): 429-434.
241. Zannad F, Alla F, Dousset B, Perez A, Pitt B. Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the Randomized Aldactone Evaluation Study (RALES). Circulation. 2000; 102(22): 2700-2706.
242. Mak GJ, Ledwidge MT, Watson CJ, Phelan DM, Dawkins IR, Murphy NF, et al. Natural history of markers of collagen turnover in patients with early diastolic dysfunction and impact of eplerenone. Journal of the American College of Cardiology. 2009; 54(18): 1674-1682.
243. Dorrance AM. Are macrophages the foot soldiers in the war waged by aldosterone against the heart? Hypertension. 2009; 54(3): 451-453.
244. Pitt B, Pitt GS. Added benefit of mineralocorticoid receptor blockade in the primary prevention of sudden cardiac death. Circulation. 2007; 115(23): 2976-2982.
245. Wagner M, Rudakova E, Volk T. Aldosterone-induced changes in the cardiac L-type Ca2+ current can be prevented by antioxidants in vitro and are absent in rats on low salt diet. Pflügers Archiv - European Journal of Physiology. 2008;457 (2): 339-349.
246. Bénitah JP, Perrier E, Gómez AM, Vassort G. Effects of aldosterone on transient outward K+ current density in rat ventricular myocytes. Journal of Physiology. 2004; 537(1): 151-160.
247. Bénitah JP, Vassort G. Aldosterone upregulates Ca2+ current in adult rat cardiomyocytes. Circulation Research. 1999; 85(12): 1139-1145.
248. Perrier E, Kerfant BG, Lalevee N, Bideaux P, Rossier MF, Richard S, et al. Mineralocorticoid receptor antagonism prevents the electrical remodeling that precedes cellular hypertrophy after myocardial infarction. Circulation. 2004; 110(7): 776-783.
249. Yee KM, Pringle SD, Struthers AD. Circadian variation in the effects of aldosterone

blockade on heart rate variability and QT dispersion in congestive heart failure. Journal of the American College of Cardiology. 2001; 37(7): 1800-1807.

250. Barr CS, Lang CC, Hanson J, Arnott M, Kennedy N, Struthers AD. Effects of adding spironolactone to an angiotensin-converting enzyme inhibitor in chronic congestive heart failure secondary to coronary artery disease. The American Journal of Cardiology. 1995; 76(17): 1259-1265.
251. Pitt B, White H, Nicolau J, Martinez F, Gheorghiade M, Aschermann M, et al. Eplerenone reduces mortality 30 days after randomization following acute myocardial infarction in patients with left ventricular systolic dysfunction and heart failure. Journal of the American College of Cardiology. 2005; 46(3): 425-431.
252. Kino T, Stauber RH, Resau JH, Pavlakis GN, Chrousos GP. Pathologic human GR mutant has a transdominant negative effect on the wild-type GR by inhibiting its translocation into the nucleus: importance of the ligand-binding domain for intracellular GR trafficking. Journal of Clinical Endocrinology and Metabolism. 2001; 86(11): 5600-5608.
253. Funder JW. Mineralocorticoid receptors: distribution and activation. Heart Failure Reviews. 2005; 10(1): 15-22.
254. Hadoke PWF, Iqbal J, Walker BR. Therapeutic manipulation of glucocorticoid metabolism in cardiovascular disease. British Journal of Pharmacology. 2009; 156(5): 689-712.
255. Ferrari P. Cortisol and the renal handling of electrolytes: role in glucocorticoid-induced hypertension and bone disease. Best Practice and Research Clinical Endocrinology and Metabolism. 2003; 17(4): 575-589.
256. Radomski M, Palmer R, Moncada S. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. Proceedings of the National Academy of Sciences. 1990; 87(24): 10043-10047.
257. Simmons WW, Ungureanu-Longrois D, Smith GK, Smith TW, Kelly RA. Glucocorticoids regulate inducible nitric oxide synthase by inhibiting tetrahydrobiopterin synthesis and L-arginine transport. Journal of Biological Chemistry. 1996; 271(39): 23928-23937.
258. Wallerath T, Witte K, Schäfer SC, Schwarz PM, Prellwitz W, Wohlfart P, et al. Down-regulation of the expression of endothelial NO synthase is likely to contribute to glucocorticoid-mediated hypertension. Proceedings of the National Academy of Sciences. 1999; 96(23): 13357-13362.
259. Mangos GJ, Walker BR, Kelly JJ, Lawson JA, Webb DJ, Whitworth JA. Cortisol inhibits cholinergic vasodilatation in the human forearm. American Journal of Hypertension. 2000; 13(11): 1155-1160.
260. Takimoto K, Li DQ, Nerbonne JM, Levitan ES. Distribution, splicing and glucocorticoid-induced expression of cardiac alpha(1C) and alpha(1D) voltage-gated Ca2+ channel mRNAs. Journal of Molecular and Cellular Cardiology. 1997; 29(11): 3035-3042.
261. Wang L, Feng ZP, Duff HJ. Glucocorticoid regulation of cardiac K+ currents and L-type Ca2+ current in neonatal mice. Circulation Research. 1999; 85(2): 168-173.
262. Sainte-Marie Y, Cat A, Perrier R, Mangin L, Soukaseum C, Peuchmaur M, et al. Conditional glucocorticoid receptor expression in the heart induces atrio-ventricular block. Journal of the Federation of American Societies for Experimental Biology. 2007; 21(12): 3133-3141.
263. Rougier JS, Muller O, Berger S, Centeno G, Schütz G, Firsov D, et al. Mineralocorticoid receptor is essential for corticosteroid-induced up-regulation of L-type calcium currents

in cultured neonatal cardiomyocytes. Pflügers Archiv - European Journal of Physiology. 2008; 456(2): 407-412.

264. Spath JA, Lane DL, Lefer AM. Protective action of methylprednisolone on the myocardium during experimental myocardial ischemia in the cat. Circulation Research. 1974; 35(1): 44-51.
265. Welman E, Selwyn A, Fox K. Lysosomal and cytosolic enzyme release in acute myocardial infarction: effects of methylprednisolone. Circulation. 1979; 59(4): 730-733.
266. Hammerman H, Kloner RA, Hale S, Schoen FJ, Braunwald E. Dose-dependent effects of short-term methylprednisolone on myocardial infarct extent, scar formation, and ventricular function. Circulation. 1983;(68): 446-452.
267. Kloner R, Fishbein M, Lew H, Maroko P, Braunwald E. Mummification of the infarcted myocardium by high dose corticosteroids. Circulation. 1978; 57(1): 56-63.
268. Jung SP, Siegrist B, Wade MR, Anthony CT, Woltering EA. Inhibition of human angiogenesis with heparin and hydrocortisone. Angiogenesis. 2001; 4(3): 175-185.
269. Small GR, Hadoke PW, Sharif I, Dover AR, Armour D, Kenyon CJ, et al. Preventing local regeneration of glucocorticoids by 11beta-hydroxysteroid dehydrogenase type 1 enhances angiogenesis. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102(34): 12165-12170.
270. Weir RAP, Tsorlalis IK, Steedman T, Dargie HJ, Fraser R, McMurray JJ, et al. Aldosterone and cortisol predict medium-term left ventricular remodelling following myocardial infarction. European Journal of Heart Failure. 2011; 13(12): 1305-1313.
271. Mihailidou AS, Loan Le TY, Mardini M, Funder JW. Glucocorticoids activate cardiac mineralocorticoid receptors during experimental myocardial infarction. Hypertension. 2009; 54(6): 1306-1312.
272. Struthers A. Aldosterone escape during ACE inhibitor therapy in chronic heart failure. European Heart Journal. 1995; 16(suppl N): 103-106.
273. Bomback AS, Klemmer PJ. The incidence and implications of aldosterone breakthrough. Nature Clinical Practice Nephrology. 2007; 3(9): 486-492.
274. Struthers AD. The clinical implications of aldosterone escape in congestive heart failure. European Journal of Heart Failure. 2004; 6(5): 539-545.
275. Padmanabhan N, Jardine AG, McGrath JC, Connell JM. Angiotensin-converting enzyme-independent contraction to angiotensin I in human resistance arteries. Circulation. 1999; 99(22): 2914-2920.
276. Petrie MC, Padmanabhan N, McDonald JE, Hillier C, Connell JM, McMurray JJ. Angiotensin converting enzyme (ACE) and non-ACE dependent angiotensin II generation in resistance arteries from patients with heart failure and coronary heart disease. Journal of the American College of Cardiology. 2001; 37(4): 1056-1061.
277. Harada E, Yoshimura M, Yasue H, Nakagawa O, Nakagawa M, Harada M, et al. Aldosterone induces angiotensin-converting-enzyme gene expression in cultured neonatal rat cardiocytes. Circulation. 2001; 104(2): 137-139.
278. Zardini P, Coats AJ, Anker SD, Graziani M, Bonizzato A, Cabrini G, et al. Failure of aldosterone suppression despite angiotensin-converting enzyme (ACE) inhibitor administration in chronic heart failure is associated with ACE DD genotype. Journal of the American College of Cardiology. 2001; 37(7): 1808-1812.
279. Cicoira M, Zanolla L, Franceschini L, Rossi A, Golia G, Zeni P, et al. Relation of aldosterone “escape” despite angiotensin-converting enzyme inhibitor administration to impaired exercise capacity in chronic congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. American Journal of Cardiology. 2002; 89(4): 403-407.

280. Sato A, Saruta T. Aldosterone escape during angiotensin-converting enzyme inhibitor therapy in essential hypertensive patients with left ventricular hypertrophy. Journal of International Medical Research. 2001; 29(1): 13-21.
281. Duprez D, De Buyzere M, Rietzschel E, Taes Y, Clement D, Morgan D, et al. Inverse relationship between aldosterone and large artery compliance in chronically treated heart failure patients. European Heart Journal. 1998; 19(9): 1371-1376.
282. Freel EM, Tsorlalis IK, Lewsey JD, Latini R, Maggioni AP, Solomon S, et al. Aldosterone status associated with insulin resistance in patients with heart failure—data from the ALOFT study. Heart. 2009; 95(23): 1920-1924.
283. Rouleau JL, Packer M, Moyé L, de Champlain J, Bichet D, Klein M, et al. Prognostic value of neurohumoral activation in patients with an acute myocardial infarction: effect of captopril. Journal of the American College of Cardiology. 1994; 24(3): 583-591.
284. Vantrimpont P, Rouleau J, Ciampi A, Harel F, De Champlain J, Bichet D, et al. Two-year time course and significance of neurohumoral activation in the Survival and Ventricular Enlargement (SAVE) Study. European Heart Journal. 1998; 19(10): 1552-1563.
285. Latini R, Masson S, Anand I, Salio M, Hester A, Judd D, et al. The comparative prognostic value of plasma neurohormones at baseline in patients with heart failure enrolled in Val-HeFT. European Heart Journal. 2004; 25(4): 292-299.
286. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. New England Journal of Medicine. 1999; 341(10): 709-717.
287. Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, et al. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. New England Journal of Medicine. 2003; 348(14): 1309-1321.
288. Rousseau MF, Gurné O, Duprez D, Van Mieghem W, Robert A, Ahn S, et al. Beneficial neurohormonal profile of spironolactone in severe congestive heart failure: results from the RALES neurohormonal substudy. Journal of the American College of Cardiology. 2002; 40(9): 1596-1601.
289. Gao X, Peng L, Adhikari CM, Lin J, Zuo Z. Spironolactone reduced arrhythmia and maintained magnesium homeostasis in patients with congestive heart failure. Journal of Cardiac Failure. 2007; 13(3): 170-177.
290. Iraqi W, Rossignol P, Angioi M, Fay R, Nuee J, Ketelslegers JM, et al. Extracellular cardiac matrix biomarkers in patients with acute myocardial infarction complicated by left ventricular dysfunction and heart failure: insights from the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) Study. Circulation. 2009; 119(18): 2471-2479.
291. Zannad F, McMurray JJ, Krum H, van Veldhuisen DJ, Swedberg K, Shi H, et al. Eplerenone in patients with systolic heart failure and mild symptoms. New England Journal of Medicine. 2011; 364(1): 1121.
292. Desai AS, Lewis EF, Li R, Solomon SD, Assmann SF, Boineau R, et al. Rationale and design of the treatment of preserved cardiac function heart failure with an aldosterone antagonist trial: a randomized, controlled study of spironolactone in patients with symptomatic heart failure and preserved ejection fraction. American Heart Journal. 2011;162 (6): 966-972.
293. White PC, Slutsker L. Haplotype analysis of CYP11B2. Endocrine Research. 1995; 21(1-2): 437-442.
294. Connell J, Fraser R, Davies E. Disorders of mineralocorticoid synthesis. Best Practice and Research Clinical Endocrinology and Metabolism. 2001; 15(1): 43-60.

295. Hautanen A, Lankinen L, Kupari M, Jänne O, Adlercreutz H, Nikkilä H, et al. Associations between aldosterone synthase gene polymorphism and the adrenocortical function in males. Journal of Internal Medicine. 2002; 243(7): 11-18.
296. Lala DS, Rice DA, Parker KL. Steroidogenic factor-1, a key regulator of steroidogenic enzyme expression, is the mouse homolog of fushi-tarazu-factor-1. Molecular Endocrinology. 1992; 6(8): 1249-1258.
297. Bassett M, Zhang Y, Clyne C, White P, Rainey W. Differential regulation of aldosterone synthase and 11beta-hydroxylase transcription by steroidogenic factor-1. Journal of Molecular Endocrinology. 2002; 28(2): 125-135.
298. Davies E, Holloway CD, Ingram MC, Inglis GC, Friel EC, Morrison C, et al. Aldosterone excretion rate and blood pressure in essential hypertension are related to polymorphic differences in the aldosterone synthase gene CYP11B2. Hypertension. 1999; 33(2): 703-707.
299. Davies E, Holloway CD, Ingram MC, Friel EC, Inglis GC, Swan L, et al. An influence of variation in the aldosterone synthase gene (CYP11B2) on corticosteroid responses to ACTH in normal human subjects. Clinical Endocrinology. 2002; 54(6): 813-817.
300. Paillard F, Chansel D, Brand E, Benetos A, Thomas F, Czekalski S, et al. Genotype-phenotype relationships for the renin-angiotensin-aldosterone system in a normal population. Hypertension. 1999; 34(3): 423-429.
301. Barbato A, Russo P, Siani A, Folkerd EJ, Miller MA, Venezia A, et al. Aldosterone synthase gene (CYP11B2) C-344T polymorphism, plasma aldosterone, renin activity and blood pressure in a multi-ethnic population. Journal of Hypertension. 2004; 22(10):. 1895-1901.
302. Lim PO, Macdonald TM, Holloway C, Friel E, Anderson NH, Dow E, et al. Variation at the aldosterone synthase (CYP11B2) locus contributes to hypertension in subjects with a raised aldosterone-to-renin ratio. Journal of Clinical Endocrinology and Metabolism. 2002; 87(9): 4398-4402.
303. Schunkert H, Hengstenberg C, Holmer SR, Broeckel U, Luchner A, Muscholl MW, et al. Lack of association between a polymorphism of the aldosterone synthase gene and left ventricular structure. Circulation. 1999; 99(17): 2255-2260.
304. Pojoga L, Gautier S, Blanc H, Guyene TT, Poirier O, Cambien F, et al. Genetic determination of plasma aldosterone levels in essential hypertension. American Journal of Hypertension. 1998; 11(7): 856-860.
305. Nicod J, Bruhin D, Auer L, Vogt B, Frey FJ, Ferrari P. A biallelic gene polymorphism of CYP11B2 predicts increased aldosterone to renin ratio in selected hypertensive patients. Journal of Clinical Endocrinology and Metabolism. 2003; 88(6): 2495-2500.
306. Imrie H, Freel M, Mayosi BM, Davies E, Fraser R, Ingram M, et al. Association between aldosterone production and variation in the 11beta-hydroxylase (CYP11B1) gene. Journal of Clinical Endocrinology and Metabolism. 2006; 91(12): 5051-5056.
307. Isaji M, Mune T, Takada N, Yamamoto Y, Suwa T, Morita H, et al. Correlation between left ventricular mass and urinary sodium excretion in specific genotypes of CYP11B2. Journal of Hypertension. 2005; 23(6): 1149-1157.
308. Tanahashi H, Mune T, Takahashi Y, Isaji M, Suwa T, Morita H, et al. Association of Lys173Arg polymorphism with CYP11B2 expression in normal adrenal glands and aldosterone-producing adenomas. Journal of Clinical Endocrinology and Metabolism. 2005; 90(11): 6226-6231.
309. Fardella C, Rodriguez H, Montero J, Zhang G, Vignolo P, Rojas A, et al. Genetic variation in P450c11AS in Chilean patients with low renin hypertension. Journal of Clinical Endocrinology and Metabolism. 1996; 81(12): 4347-4351.

310. Inglis GC, Ingram MC, Holloway CD, Swan L, Birnie D, Hillis WS, et al. Familial pattern of corticosteroids and their metabolism in adult human subjects-the Scottish Adult Twin Study. Journal of Clinical Endocrinology and Metabolism. 1999; 84(11): 4132-4137.
311. Freel E, Ingram M, Friel E, Fraser R, Brown M, Samani N, et al. Phenotypic consequences of variation across the aldosterone synthase and 11beta-hydroxylase locus in a hypertensive cohort: data from the MRC BRIGHT Study. Clinical Endocrinology. 2007; 67(6): 832-838.
312. Keavney B, Mayosi B, Gaukrodger N, Imrie H, Baker M, Fraser R, et al. Genetic variation at the locus encompassing 11beta-hydroxylase and aldosterone synthase accounts for heritability in cortisol precursor (11-deoxycortisol) urinary metabolite excretion. Journal of Clinical Endocrinology and Metabolism. 2005; 90(2): 1072-1077.
313. Ganapathipillai S, Laval G, Hoffmann IS, Castejon AM, Nicod J, Dick B, et al. CYP11B2-CYP11B1 haplotypes associated with decreased 11beta-hydroxylase activity. Journal of Clinical Endocrinology Metabolism. 2005; 90(2): 1220-1225.
314. Barr M, MacKenzie SM, Friel EC, Holloway CD, Wilkinson DM, Brain NJ, et al. Polymorphic variation in the 11beta-hydroxylase gene associates with reduced 11-hydroxylase efficiency. Hypertension. 2007; 49(1): 113-119.
315. McNamara DM, Tam SW, Sabolinski ML, Tobelmann P, Janosko K, Taylor AL, et al. Aldosterone synthase promoter polymorphism predicts outcome in African Americans with heart failure: results from the A-HeFT Trial. Journal of the American College of Cardiology. 2006; 48(6): 1277-1282.
316. Taylor AL, Ziesche S, Yancy C, Carson P, D'Agostino R Jr, Ferdinand K, et al. Combination of isosorbide dinitrate and hydralazine in blacks with heart failure. New England Journal of Medicine. 2004; 351(20): 2049-2057.
317. Baird T, Palmer B, Frampton C, Yandle T, Skelton L, Richards A, et al. Association of the aldosterone synthase gene C-344T polymorphism with risk factors and survival in a post-myocardial infarction cohort. Journal of Human Hypertension. 2007; 21(3): 256-258.
318. Dorrian C, Toole B, Alvarez-Madrazo S, Kelly A, Connell J, Wallace A. A screening procedure for primary aldosteronism based on the Diasorin Liaison® automated chemiluminescent immunoassay for direct renin. Annals of Clinical Biochemistry. 2010; 47(3): 195-199.
319. Vogelzangs N, Beekman ATF, Milaneschi Y, Bandinelli S, Ferrucci L, Penninx BW. Urinary cortisol and six-year risk of all-cause and cardiovascular mortality. Journal of Clinical Endocrinology and Metabolism. 2010; 95(11): 4959-4964.
320. Fraser R, Ingram MC, Anderson NH, Morrison C, Davies E, Connell JM. Cortisol effects on body mass, blood pressure, and cholesterol in the general population. Hypertension. 1999; 33(6): 1364-1368.
321. Stowasser M, Gordon RD. Aldosterone assays: an urgent need for improvement. Clinical Chemistry. 2006; 52(9): 1640-1642.
322. Schirpenbach C, Seiler L, Maser-Gluth C, Beuschlein F, Reincke M, Bidlingmaier M. Automated chemiluminescence-immunoassay for aldosterone during dynamic testing: comparison to radioimmunoassays with and without extraction steps. Clinical Chemistry. 2006; 52(9): 1749-1755.
323. Vogeser M, Parhofer K. Liquid chromatography tandem-mass spectrometry (LC-MS/MS)-technique and applications in endocrinology. Experimental and Clinical Endocrinology and Diabetes. 2007; 115(9): 559-570.
324. Ryoyu T, Isamu M, Masatoshi I, Hideo K, Yoshiyu T, Shuichiro Y, et al. Circadian

rhythm of plasma aldosterone and time dependent alterations of aldosterone regulators. Journal of Steroid Biochemistry. 1984; 20(1): 321-323.

325. Portaluppi F, Bagni B, degli Uberti E, Montanari L, Cavallini R, Trasforini G, et al. Circadian rhythms of atrial natriuretic peptide, renin, aldosterone, cortisol, blood pressure and heart rate in normal and hypertensive subjects. Journal of Hypertension. 1990; 8(1): 85-95.
326. Crowley S, Hindmarsh P, Honour J, Brook C. Reproducibility of the cortisol response to stimulation with a low dose of ACTH (1-24): the effect of basal cortisol levels and comparison of low-dose with high-dose secretory dynamics. Journal of Endocrinology. 1993; 136(1): 167-172.
327. Daidoh H, Morita H, Mune T, Murayama M, Hanafusa J, Ni H, et al. Responses of plasma adrenocortical steroids to low dose ACTH in normal subjects. Clinical Endocrinology. 2008; 43(3): 311-315.
328. Park Y, Park K, Kim J, Shin C, Kim S, Lee H. Reproducibility of the cortisol response to stimulation with the low dose (1μg) of ACTH. Clinical Endocrinology. 2003; 51(2): 153-158.
329. Nicholls M, Espiner E, Donald R. Plasma aldosterone response to low dose ACTH stimulation. Journal of Clinical Endocrinology and Metabolism. 1975; 41(1): 186-188.
330. Imai M, Ogishima T, Shimada H, Ishimura Y. Effect of dietary sodium restriction on mRNA for aldosterone synthase cytochrome P-450 in rat adrenals. Journal of Biochemistry. 1992; 111(4): 440-443.
331. Kathiresan S, Larson MG, Benjamin EJ, Corey D, Murabito JM, Fox CS, et al. Clinical and genetic correlates of serum aldosterone in the community: the Framingham Heart Study. American Journal of Hypertension. 2005; 18(5): 657-665.
332. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011 (WHO/NMH/NHD/MNM/11.1) (http://www.who.int/vmnis/indicators/haemoglobin.pdf, accessed [26.05.2013]).
333. Adams KF Jr, Fonarow GC, Emerman CL, LeJemtel TH, Costanzo MR, Abraham WT, et al. Characteristics and outcomes of patients hospitalized for heart failure in the United States: rationale, design, and preliminary observations from the first 100,000 cases in the Acute Decompensated Heart Failure National Registry (ADHERE). American Heart Journal. 2005; 149(2): 209-216.
334. Baldasseroni S, Opasich C, Gorini M, Lucci D, Marchionni N, Marini M, et al. Left bundle-branch block is associated with increased 1-year sudden and total mortality rate in 5517 outpatients with congestive heart failure: a report from the Italian network on congestive heart failure. American Heart Journal. 2002; 143(3): 398-405.
335. Nieminen MS, Brutsaert D, Dickstein K, Drexler H, Follath F, Harjola VP, et al. EuroHeart Failure Survey II (EHFS II): a survey on hospitalized acute heart failure patients: description of population. European Heart Journal. 2006; 27(22): 2725-2736.
336. Missov E, Calzolari C, Pau B. Circulating cardiac troponin I in severe congestive heart failure. Circulation. 1997; 96(9): 2953-2958.
337. Sato Y, Takatsu Y, Kataoka K, Yamada T, Taniguchi R, Sasayama S, et al. Serial circulating concentrations of c-reactive protein, interleukin (il)-4, and il-6 in patients with acute left heart decompensation. Clinical Cardiology. 2009; 22(12): 811-813.
338. Mueller C, Laule-Kilian K, Christ A, Brunner-La Rocca HP, Perruchoud AP. Inflammation and long-term mortality in acute congestive heart failure. American Heart Journal. 2006; 151(4): 845-850.
339. Aronson D, Burger AJ. Neurohormonal prediction of mortality following admission for

decompensated heart failure. American Journal of Cardiology. 2003; 91(2): 245-247.

340. Gheorghiade M, Pang PS, Ambrosy AP, Lan G, Schmidt P, Filippatos G, et al. A comprehensive, longitudinal description of the in-hospital and post-discharge clinical, laboratory, and neurohormonal course of patients with heart failure who die or are re-hospitalized within 90 days: analysis from the EVEREST trial. Heart Failure Reviews. 2012; 17(3): 485-509.
341. Schunkert H, Ingelfinger JR, Jacob H, Jackson B, Bouyounes B, Dzau VJ. Reciprocal feedback regulation of kidney angiotensinogen and renin mRNA expressions by angiotensin II. American Journal of Physiology - Endocrinology And Metabolism. 1992; 263(5): E863-E869.
342. Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, Stowasser M, et al. Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. Journal of Clinical Endocrinology and Metabolism. 2008; 93(9): 3266-3281.
343. Kurz RW, Stockenhuber F, Gremmel F, Graninger W. Serum concentrations of immune parameters during acute cardiogenic pulmonary edema. Critical Care Medicine. 1992; 20: 1235-1235.
344. Prakash R, Parmley WW, Horvat M, Swan H. Serum cortisol, plasma free fatty acids, and urinary catecholamines as indicators of complications in acute myocardial infarction. Circulation. 1972; 45(4): 736-745.
345. Abraham WT, Fonarow GC, Albert NM, Stough WG, Gheorghiade M, Greenberg BH, et al. Predictors of in-hospital mortality in patients hospitalized for heart failure: insights from the Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure (OPTIMIZE-HF). Journal of the American College of Cardiology. 2008; 52(5): 347-356.
346. Solomon SD, Dobson J, Pocock S, Skali H, McMurray JJ, Granger CB, et al. Influence of nonfatal hospitalization for heart failure on subsequent mortality in patients with chronic heart failure. Circulation. 2007; 116(13): 1482-1487.
347. Felker GM, Leimberger JD, Califf RM, Cuffe MS, Massie BM, Adams KF Jr, et al. Risk stratification after hospitalization for decompensated heart failure. Journal of cardiac failure. 2004; 10(6): 460-466.
348. McMurray JJ, Pitt B, Latini R, Maggioni AP, Solomon SD, Keefe DL, et al. Effects of the oral direct renin inhibitor aliskiren in patients with symptomatic heart failure. Circulation Heart Failure. 2008; 1(1): 17-24.
349. Turpeinen U, Hämäläinen E, Stenman UH. Determination of aldosterone in serum by liquid chromatography-tandem mass spectrometry. Journal of Chromatography B. 2008; 862(1): 113-118.
350. Amar L, Azizi M, Menard J, Peyrard S, Watson C, Plouin PF. Aldosterone synthase inhibition with LCI699: a proof-of-concept study in patients with primary aldosteronism. Hypertension. 2010; 56(5): 831-838.
351. Calhoun DA, White WB, Krum H, Guo W, Bermann G, Trapani A, et al. Effects of a novel aldosterone synthase inhibitor for treatment of primary hypertension: results of a randomized, double-blind, placebo-and active-controlled phase 2 Trial. Circulation. 2011; 124(18): 1945-1955.
352. Alvarez-Madrazo S, Padmanabhan S, Mayosi BM, Watkins H, Avery P, Wallace AM, et al. Familial and phenotypic associations of the aldosterone renin ratio. Journal of Clinical Endocrinology and Metabolism. 2009; 94(11): 4324-4333.
353. Masson S, Solomon S, Angelici L, Latini R, Anand IS, Prescott M, et al. Elevated plasma renin activity predicts adverse outcome in chronic heart failure, independently of

pharmacologic therapy: data from the Valsartan Heart Failure Trial (Val-HeFT). Journal of Cardiac Failure. 2010; 16(12): 964-970.

354. Tsutamoto T, Sakai H, Tanaka T, Fujii M, Yamamoto T, Wada A, et al. Comparison of active renin concentration and plasma renin activity as a prognostic predictor in patients with heart failure. Circulation Journal. 2007; 71(6): 915-921.
355. Swedberg K. Importance of neuroendocrine activation in chronic heart failure. Impact on treatment strategies. European Journal of Heart Failure. 2000; 2(3): 229-233.
356. Swedberg K, Kjekshus J. Effects of enalapril on mortality in severe congestive heart failure: results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). American Journal of Cardiology. 1988; 62(2): 60A-66A.
357. Brown JJ, Davies DL, Johnson VW, Lever AF, Robertson JI. Renin relationships in congestive cardiac failure, treated and untreated. American Heart Journal. 1970; 80(3): 329 -342.
358. Broqvist M, Dahlstrom U, Karlberg BE, Karlsson E, Marklund T. Neuroendocrine response in acute heart failure and the influence of treatment. European Heart Journal. 1989; 10(12): 1075-1083.
359. Damman K, Navis G, Smilde TD, Voors AA, van der Bij W, van Veldhuisen DJ, et al. Decreased cardiac output, venous congestion and the association with renal impairment in patients with cardiac dysfunction. European Journal of Heart Failure. 2007; 9(9): 872-878.
360. Cody RJ, Ljungman S, Covit AB, Kubo SH, Sealey JE, Pondolfino K, et al. Regulation of glomerular filtration rate in chronic congestive heart failure patients. Kidney International. 1988; 34(3): 361-7.
361. Levine T, Franciosa J, Vrobel T, Cohn J. Hyponatraemia as a marker for high renin heart failure. British Heart Journal. 1982; 47(2): 161-166.
362. Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. Journal of Clinical Investigation. 2005; 115(3): 500-508.
363. Anand IS, Latini R, Florea VG, Kuskowski MA, Rector T, Masson S, et al. C-reactive protein in heart failure prognostic value and the effect of valsartan. Circulation. 2005; 112(10): 1428-1434.
364. Shah SJ, Marcus GM, Gerber IL, Mckeown BH, Vessey JC, Jordan MV, et al. High-sensitivity C-reactive protein and parameters of left ventricular dysfunction. Journal of Cardiac Failure. 2006; 12(1): 61-65.
365. Dutka DP, Puri S, Strong R, Cleland JGF. Disruption of the relationship between renin and atrial natriuretic peptide early in the course of ventricular dysfunction. European Journal of Heart Failure. 1999; 1(4): 371-377.
366. Gottlieb SS, Kukin ML, Ahern D, Packer M. Prognostic importance of atrial natriuretic peptide in patients with chronic heart failure. Journal of the American College of Cardiology. 1989; 13(7): 1534-1539.
367. Johnson W, Omland T, Hall C, Lucas C, Myking OL, Collins C, et al. Neurohormonal activation rapidly decreases after intravenous therapy with diuretics and vasodilators for class IV heart failure. Journal of the American College of Cardiology. 2002; 39(10): 1623-1629.
368. Cleland JG, Tendera M, Adamus J, Freemantle N, Polonski L, Taylor J. The perindopril in elderly people with chronic heart failure (PEP-CHF) study. European Heart Journal. 2006; 27(19): 2338-2345.
369. Yusuf S, Pfeffer MA, Swedberg K, Granger CB, Held P, McMurray JJ, et al. Effects of candesartan in patients with chronic heart failure and preserved left-ventricular ejection fraction: the CHARM-Preserved Trial. Lancet. 2003; 362(9386): 777-781.

370. Massie BM, Carson PE, McMurray JJ, Komajda M, McKelvie R, Zile MR, et al. Irbesartan in patients with heart failure and preserved ejection fraction. New England Journal of Medicine. 2008; 359(23): 2456-2467.
371. Hernandez AF, Hammill BG, O'Connor CM, Schulman KA, Curtis LH, Fonarow GC. Clinical effectiveness of beta-blockers in heart failurefindings from the OPTIMIZE-HF (organized program to initiate lifesaving treatment in hospitalized patients with heart failure) registry. Journal of the American College of Cardiology. 2009; 53(2): 184-192.
372. Ito T, Yoshimura M, Nakamura S, Nakayama M, Shimasaki Y, Harada E, et al. Inhibitory effect of natriuretic peptides on aldosterone synthase gene expression in cultured neonatal rat cardiocytes. Circulation. 2003; 107(6): 807-810.
373. Aronson D, Burger AJ. Neurohumoral activation and ventricular arrhythmias in patients with decompensated congestive heart failure. Pacing and Clinical Electrophysiology. 2003; 26(3): 703-710.
374. Duprez DA. Role of the renin-angiotensin-aldosterone system in vascular remodeling and inflammation: a clinical review. Journal of Hypertension. 2006; 24(6): 983-991.
375. Pfeffer JM, Fischer TA, Pfeffer MA. Angiotensin-converting enzyme inhibition and ventricular remodeling after myocardial infarction. Annual Review of Physiology. 1995; 57(1): 805-826.
376. Krum H, Massie B, Abraham WT, Dickstein K, Kober L, McMurray JJ, et al. Direct renin inhibition in addition to or as an alternative to angiotensin converting enzyme inhibition in patients with chronic systolic heart failure: rationale and design of the Aliskiren Trial to Minimize OutcomeS in Patients with HEart failuRE (ATMOSPHERE) study. European Journal of Heart Failure. 2011; 13(1): 107-114.
377. Gheorghiade M, Albaghdadi M, Zannad F, Fonarow GC, Böhm M, Gimpelewicz C, et al. Rationale and design of the multicentre, randomized, double-blind, placebo-controlled Aliskiren Trial on Acute Heart Failure Outcomes (ASTRONAUT). European Journal of Heart Failure. 2011; 13(1): 100-106.
378. Packer M, Califf RM, Konstam MA, Krum H, McMurray JJ, Rouleau JL, et al. Comparison of omapatrilat and enalapril in patients with chronic heart failure. Circulation. 2002; 106(8): 920-926.
379. Rouleau JL, Pfeffer MA, Stewart DJ, Isaac D, Sestier F, Kerut EK, et al. Comparison of vasopeptidase inhibitor, omapatrilat, and lisinopril on exercise tolerance and morbidity in patients with heart failure: IMPRESS randomised trial. Lancet. 2000; 356(9230): 615-620.
380. Solomon SD, Zile M, Pieske B, Voors A, Shah A, Kraigher-Krainer E, et al. The angiotensin receptor neprilysin inhibitor LCZ696 in heart failure with preserved ejection fraction: a phase 2 double-blind randomised controlled trial. Lancet. 2012;380 (9851): 1387-1395.
381. Walker BR, Phillips DI, Noon JP, Panarelli M, Andrew R, Edwards HV, et al. Increased glucocorticoid activity in men with cardiovascular risk factors. Hypertension. 1998; 31(4): 891-895.
382. Bain R, Fox J, Jagger J, Davies M, Littler W, Murray R. Serum cortisol levels predict infarct size and patient mortality. International Journal of Cardiology. 1992; 37(2): 145-150.
383. Yamaji M, Tsutamoto T, Kawahara C, Nishiyama K, Yamamoto T, Fujii M, et al. Effect of eplerenone versus spironolactone on cortisol and hemoglobin A1c levels in patients with chronic heart failure. American Heart Journal. 2010; 160(5): 915-921.
384. Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, Francis GS, et al. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure.

New England Journal of Medicine. 1984; 311(13): 819-823.

385. Farese RV Jr, Biglieri EG, Shackleton CH, Irony I, Gomez-Fontes R. Licorice-induced hypermineralocorticoidism. New England Journal of Medicine. 1991; 325(17): 1223-1227.
386. Stewart PM. Tissue-specific Cushing's syndrome, 11beta-hydroxysteroid dehydrogenases and the redefinition of corticosteroid hormone action. European Journal of Endocrinology. 2003; 149(3): 163-168.
387. Quinkler M, Zehnder D, Lepenies J, Petrelli MD, Moore JS, Hughes SV, et al. Expression of renal 11beta-hydroxysteroid dehydrogenase type 2 is decreased in patients with impaired renal function. European Journal of Endocrinology. 2005; 153(2): 291-299.
388. McKenna TJ, Island DP, Nicholson WE, Miller RB, Lacroix A, Liddle GW. ACTH stimulates late steps in cortisol biosynthesis. Acta Endocrinologica. 1979; 90(1): 122-132.
389. Kolanowski J, Ortega N, Crabbe J. Corticotropin-induced changes in enzymatic activities of the post-pregnenolone steroidogenic pathway in rabbit adrenocortical cells. Journal of Steroid Biochemistry. 1988; 29(2): 249-255.
390. Kramer R, Simpson E, Waterman M. Induction of 11beta-hydroxylase by corticotropin in primary cultures of bovine adrenocortical cells. Journal of Biological Chemistry. 1983; 258(5): 3000-3005.
391. Ulrich-Lai YM, Figueiredo HF, Ostrander MM, Choi DC, Engeland WC, Herman JP. Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. American Journal of Physiology-Endocrinology and Metabolism. 2006; 291(5): E965-E973.
392. McKenna TJ, Miller RB, Liddle GW. Plasma pregnenolone and 17-OH-pregnenolone in patients with adrenal tumors, ACTH excess, or idiopathic hirsutism. Journal of Clinical Endocrinology and Metabolism. 1977; 44(2): 231-236.
393. Ney RL, Dexter RN, Davis WW, Garren LD. A study of the mechanisms by which adrenocorticotropic hormone maintains adrenal steroidogenic responsiveness. Journal of Clinical Investigation. 1967; 46(12): 1916-1924.
394. Simpson ER, Waterman MR. Regulation of the synthesis of steroidogenic enzymes in adrenal cortical cells by ACTH. Annual Review of Physiology. 1988; 50(1): 427-440.
395. Redgate E. Role of the baroreceptor reflexes and vasoactive polypeptides in the corticotropin release evoked by hypotension. Endocrinology. 1968; 82(4): 704-720.
396. Späth-Schwalbe E, Born J, Schrezenmeier H, Bornstein S, Stromeyer P, Drechsler S, et al. Interleukin-6 stimulates the hypothalamus-pituitary-adrenocortical axis in man. Journal of Clinical Endocrinology and Metabolism. 1994; 79(4): 1212-1214.
397. Lyson K, McCann S. The effect of interleukin-6 on pituitary hormone release in vivo and in vitro. Neuroendocrinology. 1991; 54(3): 262-266.
398. Tsigos C, Chrousos GP. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. Journal of Psychosomatic Research. 2002; 53(4): 865-871.
399. Bacon G, Kenny F, Murdaugh H, Richards C. Prolonged serum half-life of cortisol in renal failure. Johns Hopkins Medical Journal. 1973; 132(2): 127-131.
400. Aronson D, Mittleman MA, Burger AJ. Elevated blood urea nitrogen level as a predictor of mortality in patients admitted for decompensated heart failure. American Journal of Medicine. 2004; 116(7): 466-473.
401. Filippatos G, Rossi J, Lloyd-Jones DM, Stough WG, Ouyang J, Shin DD, et al. Prognostic value of blood urea nitrogen in patients hospitalized with worsening heart

failure: insights from the Acute and Chronic Therapeutic Impact of a Vasopressin Antagonist in Chronic Heart Failure (ACTIV CHF) study. Journal of Cardiac Failure. 2007; 13(5): 360-364.

402. Brillon D, Zheng B, Campbell R, Matthews D. Effect of cortisol on energy expenditure and amino acid metabolism in humans. American Journal of Physiology-Endocrinology and Metabolism. 1995; 268(3): E501-E513.
403. Vergaro G, Emdin M, Iervasi A, Zyw L, Gabutti A, Poletti R, et al. Prognostic value of plasma renin activity in heart failure. American Journal of Cardiology. 2011; 108(2): 246-251.
404. Nguyen G. Renin/prorenin receptors. Kidney International. 2006; 69(9): 1503-1506.
405. Hitom H, Liu G, Nishiyama A. Role of (pro) renin receptor in cardiovascular cells from the aspect of signaling. Frontiers in Bioscience. 2010; 2: 1246-1249.
406. Shepherd J, Blauw GJ, Murphy MB, Bollen EL, Buckley BM, Cobbe SM, et al. Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. Lancet. 2002; 360(9346): 1623-1630.
407. Kennon B, Ingram MC, Friel EC, Anderson NH, MacKenzie SM, Davies E, et al. Aldosterone synthase gene variation and adrenocortical response to sodium status, angiotensin II and ACTH in normal male subjects. Clinical Endocrinology. 2004; 61(2):. 174-181.
408. Freel E, Ingram M, Wallace A, White A, Fraser R, Davies E, et al. Effect of variation in CYP11B1 and CYP11B2 on corticosteroid phenotype and hypothalamic-pituitary-adrenal axis activity in hypertensive and normotensive subjects. Clinical Endocrinology. 2008; 68(5): 700-706.
409. Connell J, Fraser R, MacKenzie SM, Friel EC, Ingram MC, Holloway CD, et al. The impact of polymorphisms in the gene encoding aldosterone synthase (CYP11B2) on steroid synthesis and blood pressure regulation. Molecular and Cellular Endocrinology. 2004; 217(1-2): 243-247.
410. Stella P, Bigatti G, Tizzoni L, Barlassina C, Lanzani C, Bianchi G, et al. Association between aldosterone synthase (CYP11B2) polymorphism and left ventricular mass in human essential hypertension. Journal of the American College of Cardiology. 2004; 43(2): 265-270.
411. Tamaki S, Iwai N, Tsujita Y, Kinoshita M. Genetic polymorphism of CYP11B2 gene and hypertension in Japanese. Hypertension. 1999; 33(1): 266-270.
412. White PC, Rainey WE. Polymorphisms in CYP11B genes and 11-hydroxylase activity. Journal of Clinical Endocrinology and Metabolism. 2005; 90(2): 1252-1255.
413. Sookoian S, Gianotti TF, González CD, Pirola CJ. Association of the C-344T aldosterone synthase gene variant with essential hypertension: a meta-analysis. Journal of Hypertension. 2007; 25(1): 5-13.
414. Kalogeropoulos A, Georgiopoulou V, Kritchevsky SB, Psaty BM, Smith NL, Newman AB, et al. Epidemiology of incident heart failure in a contemporary elderly cohort: the health, aging, and body composition study. Archives of Internal Medicine. 2009; 169(7): 708-715.
415. Flack JM, Oparil S, Pratt JH, Roniker B, Garthwaite S, Kleiman JH, et al. Efficacy and tolerability of eplerenone and losartan in hypertensive black and white patients. Journal of the American College of Cardiology. 2003; 41(7): 1148-1155.
416. Takai E, Akita H, Kanazawa K, Shiga N, Terashima M, Matsuda Y, et al. Association between aldosterone synthase (CYP11B2) gene polymorphism and left ventricular volume in patients with dilated cardiomyopathy. Heart. 2002; 88(6): 649-650.
417. Tiago AD, Badenhorst D, Skudicky D, Woodiwiss AJ, Candy GP, Brooksbank R, et al.

An aldosterone synthase gene variant is associated with improvement in left ventricular ejection fraction in dilated cardiomyopathy. Cardiovascular Research. 2002; 54(3): 584-589.

418. Biolo A, Chao T, Duhaney TAS, Kotlyar E, Allensworth-Davies D, Loscalzo J, et al. Usefulness of the aldosterone synthase gene polymorphism C-344-T to predict cardiac remodeling in African-Americans versus non-African-Americans with chronic systolic heart failure. American Journal of Cardiology. 2007; 100(2): 285-290.
419. Gheorghiade M, Pang PS. Acute heart failure syndromes. Journal of the American College of Cardiology. 2009; 53(7): 557-573.
420. De Luca L, Mebazaa A, Filippatos G, Parissis JT, Böhm M, Voors AA, et al. Overview of emerging pharmacologic agents for acute heart failure syndromes. European Journal of Heart Failure. 2008; 10(2): 201-213.
421. Yoshida M, Ma J, Tomita T, Morikawa N, Tanaka N, Masamura K, et al. Mineralocorticoid receptor is overexpressed in cardiomyocytes of patients with congestive heart failure. Congestive Heart Failure. 2007; 11(1): 12-16.
422. Ullian ME. The role of corticosteroids in the regulation of vascular tone. Cardiovascular Research. 1999; 41(1): 55-64.
423. Walker B, Williams B. Corticosteroids and vascular tone: mapping the messenger maze. Clinical Science. 1992; 82: 597-605.
424. Anker SD, McMurray JJ. Time to move on from 'time-to-first': should all events be included in the analysis of clinical trials? European Heart Journal. 2012; 33(22): 2764-2765.
425. Solomon SD, Anavekar N, Skali H, McMurray JJ, Swedberg K, Yusuf S, et al. Influence of ejection fraction on cardiovascular outcomes in a broad spectrum of heart failure patients. Circulation. 2005; 112(24): 3738-3744.